JC08 Rec'd PCT/PTO 29 JAN 2001

FORM PTO-1390 (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEYS DOCKET NO. 2618-102-PUS U.S. APPLICATION NO. (If known, see 35 CFR 1.5)
INTERNATIONAL APPLICATION NO. PCT/US99/17309	INTERNATIONAL FILING DATE 29 July 1999	PRIORITY DATE CLAIMED 29 July 1998

TITLE OF INVENTION
"T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"

APPLICANT(S) FOR DO/EO/US
SIM, Gek-Kee and DREITZ, Matthew J.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f))) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. below concern documents or information included:

11. [ ] An Information Disclosure Statement under 37n CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.23 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

"EXPRESS MAIL" MAILING LABEL NUMBER: EL686041709
DATE OF DEPOSIT: January 29, 2001

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE ASSISTANT COMMISSIONER FOR PATENTS, BOX PCT, WASHINGTON, D.C. 20231.

TYPED OR PRINTED NAME: JANICE MESSER

SIGNATURE: Janice Messer

JC02 Rec'd PCT/PTO 2 9 JAN 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/744847	INTERNATIONAL APPLICATION NO. PCT/US99/17309	ATTORNEY DOCKET NUMBER 2618-102-PUS

		CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):** Search Report has been prepared by the EPO or JPO	$860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482)	$690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid USPTO	$1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisified provisions of PCT Article 33(2)-(4)	$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 690.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 - 20 =	0	x $18.00	$ 0.00	
Independent Claims	5 - 3 =	2	x $80.00	$ 160.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $270.00	$	
	TOTAL OF ABOVE CALCULATIONS =			$ 850.00	

	CALCULATIONS	PTO USE ONLY
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).	$	
SUBTOTAL =	$	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$ 850.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 850.00	
	Amount to be: refunded	$
	charged	$

a. [X] A check in the amount of $ 850.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ______________ in the amount of $_________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-1970. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 cfr 1.494 or 1.495 has not been met, a petition to revive (37 cfr 1.137(a) or (b)) must be filed and granted to restore the application pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Gary J. Connell
Registration No. 32,020

09/744847

JC02 Rec'd PCT/PTO 2 9 JAN 2001

PATENT APPLICATIONS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:

HESKA CORPORATION

Int'l. Serial No.: PCT/US99/17309

Int'l. Filing Date: 29 July 1999

Priority Date: 29 July 1998

For: "T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"

Atty. File No.: 2618-102-PCT

PRELIMINARY AMENDMENT

"EXPRESS MAIL" MAILING LABEL NUMBER: EL686041709
DATE OF DEPOSIT: 1-29-01

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE ASSISTANT COMMISSIONER FOR PATENTS, BOX PCT, WASHINGTON, D.C. 20231.

TYPED OR PRINTED NAME: JANICE MESSER

SIGNATURE: Janice Messer

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the initial review of the above-identified patent application by the Examiner, please enter the following Preliminary Amendment. Fees for this Preliminary Amendment are calculated and included with the Transmittal Letter accompanying this Amendment. Please charge any underpayment or debit any overpayment to Deposit Account 19-1970.

Please amend the above-identified patent application as follows:

IN THE CLAIMS:

Please cancel Claims 1-48.

Please add the following new Claims 49 through 68:

49. An isolated protein comprising a protein selected from the group consisting of:

(a) an isolated protein having an amino acid sequence that is at least about 55 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 amino acids in length;

(b) an isolated protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 15 amino acids in length;

(c) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:35, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 25 amino acids in length;

(d) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:38, SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 35 amino acids in length;

(e) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(f) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment that is at least about 30 nucleotides in length;

(g) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid

sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(h) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

50. An isolated nucleic acid molecule having a nucleic acid sequence that is selected from the group consisting of:

(a) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:30 and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:33, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(c) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, SEQ ID NO:36, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte

from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:67, or a fragment thereof that is at least about 40 nucleotides in length;

(d) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:39, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length;

(e) a nucleic acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56;

(f) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30, or a fragment thereof, wherein said fragment has an at least a 20 contiguous nucleotide region identical in sequence to a 20 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30;

(g) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, and SEQ ID NO:33, or a fragment thereof, wherein said fragment has an at least a 25 contiguous nucleotide region identical in sequence to a 25 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31 and SEQ ID NO:33;

(h) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36, or a fragment thereof, wherein said fragment has an at least a 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36;

(i) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39, or a fragment thereof, wherein said fragment has an at least a 60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39;

(j) an isolated nucleic acid molecule having a nucleic acid sequence encoding a protein selected from the group consisting of:

(1) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(2) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(3) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(4) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

(k) an isolated oligonucleotide comprising a unique nucleic acid sequence within a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, and SEQ ID NO:80; and a homolog thereof.

51. A method to detect expansion of T cells in an animal comprising:

(a) identifying the presence of one or more T cell receptor nucleic acid molecule(s) having unique nucleic acid sequences within variable regions of beta chain nucleic acid molecules selected from the group consisting of nCaVβ3$_{381}$, nCaVβ4$_{408}$, nCaVβ4$_{384}$, nCaVβ12$_{408}$, nCaVβ12$_{402}$, nCaVβ72$_{438}$, nCaVβ72$_{399}$, nCaVβ3$_{333}$, nCaVβ4$_{351}$, nCaVβ12$_{339}$, nCaVβ72$_{423}$, nCaVβ21$_{396}$, nCaVβ54$_{354}$ and nCaVβ182$_{369}$ by forming detectable products; and

(b) detecting the expansion of said T cells by determining production of said product.

52. A therapeutic composition that, when administered to an animal, regulates an immune response in said animal, said therapeutic composition comprising a therapeutic compound selected from the group consisting of:

(i) an isolated protein comprising a TCR Vβ protein selected from the group consisting of:

(a) an isolated protein having an amino acid sequence that is at least about 55 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 amino acids in length;

(b) an isolated protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 15 amino acids in length;

(c) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:35, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 25 amino acids in length; and

(d) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:38, SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 35 amino acids in length;

(ii) a mimetope of any of said TCR Vβ proteins;

(iii) a chimeric form of any of said TCR Vβ proteins;

(iv) an isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:30 and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:33, and a nucleic acid sequence that encodes an

amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(c) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, SEQ ID NO:36, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:67, or a fragment thereof that is at least about 40 nucleotides in length;

(d) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:39, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length; and

(e) a nucleic acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56;

(v) an isolated antibody that selectively binds to any of said TCR Vβ proteins; and

(vi) an inhibitor of TCR Vβ protein activity identified by its ability to inhibit the activity of said TCR Vβ proteins.

53. A method to produce a TCR Vβ protein, said method comprising culturing a cell capable of expressing said protein, said protein selected from the group consisting of:

(a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected

from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment that is at least about 30 nucleotides in length;

(c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

54. The protein of Claim 49, wherein said protein, when administered to an animal, can perform a function selected from the group consisting of eliciting an immune response against a TCR Vβ protein and binding to a MHC molecule that binds to a TCR Vβ protein.

55. The nucleic acid molecule of Claim 50, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a TCR Vβ protein.

56. The nucleic acid molecule of the Claim 50, wherein said nucleic acid molecule encodes a protein that elicits an immune response against a naturally-occurring TCR Vβ protein.

57. A recombinant molecule comprising a nucleic acid molecule as set forth in Claim 50 operatively linked to a transcription control sequence.

58. A recombinant virus comprising a nucleic acid molecule as set forth in Claim 50.

59. A recombinant cell comprising a nucleic acid molecule as set forth in Claim 50.

60. The nucleic acid of Claim 50, wherein said nucleic acid molecule comprises an oligonucleotide from about 15 nucleotides to about 25 nucleotides in length.

61. The method of Claim 51, wherein said step of detecting comprises comparing formation of one detectable product with one or more other detectable products.

62. The method of Claim 51, wherein said variable region has a nucleic acid sequence selected from the group consisting of SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

63. The method of Claim 51, wherein said step of identification comprises:

(a) contacting a sample containing DNA from T cells with a reagent having specificity for one of said unique nucleic acid sequences; and

(b) determining the presence of DNA carrying said unique nucleic acid sequence.

64. A composition comprising the protein of Claim 49, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant and a carrier.

65. A composition comprising the nucleic acid of Claim 50, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant and a carrier.

66. A method to regulate an immune response in an animal comprising administering to the animal a therapeutic composition comprising the protein of Claim 49.

67. A method to regulate an immune response in an animal comprising administering to the animal a therapeutic composition comprising the nucleic acid of Claim 50.

68. A method for prescribing treatment for specific disease, comprising:

(a) identifying the presence of a T cell receptor nucleic acid molecule having a unique nucleic acid sequence within a variable region of a beta chain nucleic acid molecule selected from the group consisting of $nCaV\beta3_{333}$, $nCaV\beta4_{351}$, $nCaV\beta12_{339}$, $nCaV\beta72_{423}$, $nCaV\beta21_{396}$,

$nCaV\beta54_{354}$, $nCaV\beta182_{369}$, $nCaV\beta3_{381}$, $nCaV\beta4_{408}$, $nCaV\beta12_{408}$, $nCaV\beta72_{438}$, $nCaV\beta21_{462}$, $nCaV\beta54_{417}$ and $nCaV\beta182_{423}$; and

(b) prescribing a treatment comprising administering to said animal a therapeutic composition comprising the invention of Claim 1.

REMARKS

The above amendments are being submitted in connection with the national stage filing of the present Application. The amendments eliminate the multiple dependent claims from the Application.

Respectfully submitted,

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"T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"

FIELD OF THE INVENTION

The present invention relates to T cell receptor beta chain variable region nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and inhibitors of such proteins or nucleic acid molecules. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to regulate an immune response in an animal.

BACKGROUND OF THE INVENTION

The immune system of an animal is characterized by its ability to respond to a diverse set of antigenic determinants, or epitopes. This response is reflected through T and B lymphocytes, also referred to as T cells and B cells, respectively. The immune system, comprising these specialized cells, recognizes and processes foreign pathogens and macromolecules. Lymphocytes individually exhibit high specificity in recognition of particular molecular structures of antigens. The structural properties are recognized by T cell receptors, which act as antigen receptors.

T cell receptors (TCR) are members of the immunoglobulin superfamily. A TCR molecule comprises two polypeptide chains, generally an alpha chain and a beta chain. Each chain comprises an amino terminal variable region domain (V) and a carboxyl terminal constant region domain (C), and can be designated with α or β when indicating the particular chain of origin. V and C regions are encoded by V region or C region genes, respectively. Each domain can be stabilized by a disulfide bond between two conserved cysteine residue pairs on each chain. Each chain is anchored to the cell membrane by a hydrophobic transmembrane domain, which typically spans the entire lipid bilayer of the membrane. A short carboxyl domain extends into the cytoplasm.

The α and β chains of the TCR are encoded by gene segments analogous to the variable region (V), the diversity region (D), the joining region (J) and the constant region (C) of immunoglobulin genes. Diversity in the TCR repertoire arises, in part,

from the rearrangement of V, D and J regions and from the insertion of or deletion of nucleotides at the junction between the V, D and J regions.

Previous investigators have described TCR beta chain sequences, e.g., Malisson et al., *Cell*, vol. 37, pp. 1101-1110, 1984; Patten et al., *Nature*, vol. 312, pp. 40-46, 1984; Davis et al., *Nature*, vol. 334, pp. 395-402, 1988; Hood et al., U.S. Patent No. 4,886,743, issued Dec. 12, 1989; and Makrides et al., U.S. Patent No. 5,552,300, issued Sep. 3, 1996.

T cells play a pivotal role in the differentiation and regulation of immune cells. Previous investigators have studied diseases in which there appears to be improper immune regulation, such as autoimmunity and some forms of immunodeficiency, and have implicated T cells in the pathogenesis of such diseases. In addition, situations exist in which clonal or oligoclonal expansion of a particular T cell population, identified by the presence of a particular TCR, can be representative of a disease state. One example is the presence of malignancy which has resulted in a T cell leukemia or lymphoma (e.g., Hood et al., *ibid.*). In situations of T cell leukemias or lymphomas, a TCR acts as a unique tumor marker since the TCR is stably rearranged and presented on the surface of the cell.

In summary, there remains a need to develop methods and compounds useful for the detection and treatment of undesired immune responses involving T cells.

SUMMARY OF THE INVENTION

The present invention relates to T cell receptor nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and inhibitors of such proteins or nucleic acid molecules. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to regulate an immune response in an animal. The present invention also includes methods to detect T cell expansion using reagents including or derived from such nucleic acid molecules, proteins and/or antibodies, as well as the use of such methods to diagnose abnormal states or disease in an animal.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates PCR amplified TCR Vβ DNA using DNA from tissue of normal dogs and dogs having lymphoma.

Figs. 2A, 2B and 2C illustrate fingerprints of TCR Vβ DNA from a normal dog.

Figs. 3A, 3B and 3C illustrate fingerprints of TCR Vβ DNA from a dog having lymphoma.

Fig. 4 illustrates a comparison between fingerprints of TCR Vβ DNA from a normal dog and a dog having lymphoma.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated T cell receptor beta chain variable region (TCR Vβ) proteins, isolated TCR Vβ nucleic acid molecules, antibodies directed against TCR Vβ proteins, and compounds derived therefrom that regulate the immune response of an animal. A TCR Vβ protein can refer to a TCR Vβ protein or a homolog thereof. As used herein, the term "TCR Vβ" refers to a molecule that can include the variable (V) region, alone or in combination with the diversity (D) and/or joining (J) regions of a TCR beta chain. It is known to one of skill in the art that the size and sequence of V, D and J regions of a TCR beta chain can vary as a result of any given recombination event between genes encoding such V, D and J regions. Typical consensus sequences used to identify the junction between the V, D and J regions are also known to one of skill in the art, thereby enabling the identification of the size and sequence of the V, D or J regions of a TCR beta chain from a novel nucleic acid or amino acid sequence. Compounds derived from TCR Vβ proteins or nucleic acid molecules of the present invention include compounds including at least a portion of, or designed using, such proteins or nucleic acid molecules.

As used herein, the phrase "regulate an immune response" refers to modulating the activity of cells involved in an immune response. The term "regulate" can refer to increasing or decreasing an immune response Regulation of an immune response can be determined using methods known in the art as well as methods disclosed herein. As used herein, the terms isolated TCR Vβ proteins and isolated TCR Vβ nucleic acid molecules refers to TCR Vβ proteins and TCR Vβ nucleic acid molecules derived from mammals, preferably canids, more preferably dogs, and, as such, can be obtained from

their natural source, or can be produced using, for example, recombinant nucleic acid technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies, and compounds derived therefrom as therapeutic compositions to regulate the immune response of an animal as well as in other applications, such as those disclosed below.

One embodiment of the present invention is an isolated protein that includes a TCR Vβ protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms “comprising”, “including”, and “having” can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

As used herein, an isolated TCR Vβ protein of the present invention (i.e., a TCR Vβ protein) can be a full-length protein or any homolog of such a protein. Full-length proteins can refer to proteins having the V, D, J and C regions of a beta chain or one or more of such regions. It is to be noted that the term "a homolog" refers to one or more or at least one homolog. An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein’s ability to elicit an immune response against a TCR Vβ protein or to bind to a major histocompatability (MHC) molecule or superantigen. Examples of TCR Vβ homologs include TCR Vβ proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of eliciting an immune response against a TCR Vβ protein, and/or of binding to an antibody directed against a TCR Vβ protein. That is, when the homolog is administered to an animal as an immunogen, using techniques

known to those skilled in the art, the animal will produce an immune response against at least one epitope of a natural TCR Vβ protein. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about six to seven amino acids. Other examples of TCR Vβ protein homologs include those homologs that are capable of binding to MHC, in the presence or absence of peptide, or superantigen. The ability of a protein to bind to MHC or superantigen can be measured using various methods well known to those of skill in the art.

TCR Vβ protein homologs can be the result of natural allelic variation or natural mutation. TCR Vβ protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

TCR Vβ proteins of the present invention are encoded by TCR Vβ nucleic acid molecules. As used herein, a TCR Vβ nucleic acid molecule includes nucleic acid sequences related to a natural TCR Vβ gene. It is to be noted that the term "a nucleic acid molecule", "a gene" or "a nucleic acid sequence" refers to one or more or at least one nucleic acid molecule, gene or nucleic acid sequence, respectively. As used herein, a TCR Vβ gene includes all regions of the gene such as regulatory regions that control production of the TCR Vβ protein encoded by the gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that coding region that is translated into a full-length, i.e., a complete, protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:1, as well as the complement of SEQ ID NO:1. Nucleic acid sequence SEQ ID NO:1 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nCaVβ3_{381}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaVβ3_{381} comprises the coding region for the V, D and J regions of TCRVβ3 (also referred to herein as hcVβ3). The complement of SEQ ID NO:1 (represented herein by SEQ ID NO:3) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:1, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is complementary to (i.e., can form a double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:1 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding a TCR Vβ protein of the present invention.

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:4, as well as the complement of SEQ ID NO:4 represented by SEQ ID NO:6. Nucleic acid sequence SEQ ID NO:4 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nCaVβ4_{408}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaVβ4_{408} comprises the coding region for the V, D and J regions of TCRVβ4 (also referred to herein as hcVβ4).

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:9, as well as the complement of SEQ ID NO:9 represented by SEQ ID NO:11. Nucleic acid sequence SEQ ID NO:9 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nCaVβ12_{408}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaVβ12_{408} comprises the coding region for the V, D and J regions of TCRVβ12 (also referred to herein as hcVβ12).

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:98, as well as the complement of SEQ ID NO:98 represented by SEQ ID NO:100. Nucleic acid sequence SEQ ID NO:98 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule $nCaV\beta72_{438}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaV\beta72_{438}$ comprises the coding region for the V, D and J regions of TCRVβ72 (also referred to herein as hcVβ72).

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:19, as well as the complement of SEQ ID NO:19 represented by SEQ ID NO:19. Nucleic acid sequence SEQ ID NO:19 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule $nCaV\beta21_{462}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaV\beta21_{462}$ comprises the coding region for the V, D and J regions of TCRVβ21 (also referred to herein as hcVβ21).

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:22, as well as the complement of SEQ ID NO:22 represented by SEQ ID NO:22. Nucleic acid sequence SEQ ID NO:22 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule $nCaV\beta54_{417}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaV\beta54_{417}$ comprises the coding region for the V, D and J regions of TCRVβ54 (also referred to herein as dtb54).

In another embodiment, a TCR Vβ gene of the present invention includes the nucleic acid sequence SEQ ID NO:25, as well as the complement of SEQ ID NO:25 represented by SEQ ID NO:25. Nucleic acid sequence SEQ ID NO:25 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule $nCaV\beta182_{423}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaV\beta182_{423}$ comprises the coding region for the V, D and J regions of TCRVβ182 (also referred to herein as dtb182).

In another embodiment, a TCR Vβ gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, SEQ ID

NO:98, SEQ ID NO:100, or any other TCR Vβ nucleic acid sequence cited herein. An allelic variant of a TCR Vβ nucleic acid molecule including SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:98 and SEQ ID NO:100, is a nucleic acid molecule that occurs at essentially the same locus (or loci) in the genome as the nucleic acid molecule including SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:98 and SEQ ID NO:100, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given genome, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles.

In one embodiment of the present invention, an isolated TCR Vβ protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene encoding a TCR Vβ protein (i.e., to a TCR Vβ gene). The minimal size of a TCR Vβ protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the TCR Vβ nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given nucleic acid molecule.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem. 138*, 267-284. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (expressed as molarity (M), in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules longer than about 50 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

$$T_m = 81.5°C + 16.6 \log M + 0.41(\% G + C) - 500/n - 0.61(\% \text{ formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base-pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For

example, T_m decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with greater than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow about 30% base-pair mismatch (i.e., about 70% identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given nucleic acid molecule under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled probe suspended in a hybridization solution. Examples of common hybridization reaction techniques include the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under stringent hybridization conditions with a specific or known canine nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. The average G + C content of canine DNA includes about 35%, about 36%, about 37%, about 38%, about 39%, about 41%, about 42%, about 43%, about 44%, about 45%, with about 40% being preferred. The unknown nucleic acid

molecules would be attached to a support membrane, and the specified 150 bp nucleic acid molecule would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the T_m of perfect hybrids would be about 80.8°C:

$$81.5°C + 16.6 \log (.15M) + (0.41 \times 40) - (500/150) - (0.61 \times 0) = 80.8°C.$$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 50.8°C. It is within the skill of one in the art to calculate the hybridization temperature based on the formulae and G/C content disclosed herein.

In one embodiment of the present invention, a preferred TCR Vβ nucleic acid molecule includes a nucleic acid molecule which has greater than about 50 base pairs and which hybridizes under conditions which preferably allow about 20% base pair mismatch, more preferably under conditions which allow about 15% base pair mismatch, more preferably under conditions which allow about 10% base pair mismatch and even more preferably under conditions which allow about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 or SEQ ID NO:71.

Another preferred TCR Vβ nucleic acid molecule of the present invention includes a nucleic acid molecule which has greater than about 150 base pairs and which hybridizes under conditions which preferably allow about 30% base pair mismatch,

more preferably under conditions which allow about 25% base pair mismatch, more preferably under conditions which allow about 20% base pair mismatch, more preferably under conditions which allow about 15% base pair mismatch, more preferably under conditions which allow about 10% base pair mismatch and even more preferably under conditions which allow about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 or SEQ ID NO:71.

Another preferred TCR Vβ nucleic acid molecule of the present invention includes a nucleic acid molecule which has greater than about 200 base pairs and which hybridizes under conditions which preferably allow about 30% base pair mismatch, more preferably under conditions which allow about 25% base pair mismatch, more preferably under conditions which allow about 20% base pair mismatch, more preferably under conditions which allow about 15% base pair mismatch, more preferably under conditions which allow about 10% base pair mismatch and even more preferably under conditions which allow about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73 or SEQ ID NO:74.

Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein said molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 49°C, to an isolated

nucleic acid molecule selected from the group consisting of SEQ ID NO:3, SEQ ID NO:30, the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61 or SEQ ID NO:62.

Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein said molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 56°C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:33, the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:63, SEQ ID NO:64 or SEQ ID NO:65. Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein said molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 53°C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:36, the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:66, SEQ ID NO:67 or SEQ ID NO:68. Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein said molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 41°C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:39, the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:69, SEQ ID NO:70 or SEQ ID NO:71. Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein said molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 29°C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:42, the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:72, SEQ ID NO:73 or SEQ ID NO:74.

Another embodiment of the present invention includes TCR Vβ proteins. A preferred TCR Vβ protein includes a protein encoded by a nucleic acid molecule which has greater than about 50 base pairs and which hybridizes under conditions which

preferably allow about 20% base pair mismatch, more preferably under conditions which allow about 15% base pair mismatch, more preferably under conditions which allow about 10% base pair mismatch and even more preferably under conditions which allow about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.

Another preferred TCR Vβ protein of the present invention includes a protein encoded by a nucleic acid molecule which has greater than about 150 base pairs and which hybridizes under conditions which preferably allow about 30% base pair mismatch, more preferably under conditions which allow about 25% base pair mismatch, more preferably under conditions which allow about 20% base pair mismatch, more preferably under conditions which allow about 15% base pair mismatch, more preferably under conditions which allow about 10% base pair mismatch and even more preferably under conditions which allow about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.

Another preferred TCR Vβ protein of the present invention includes a protein encoded by a nucleic acid molecule which has greater than about 50 base pairs which is preferably about 80% identical, more preferably about 85% identical, more preferably

about 90% identical, and even more preferably about 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 or SEQ ID NO:71.

Yet another preferred TCR Vβ of the present invention includes a protein encoded by a nucleic acid molecule which has greater than about 150 base pairs which is preferably about 70% identical, more preferably about 75% identical, more preferably about 80% identical, more preferably about 85% identical, more preferably about 90% identical and even more preferably about 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 or SEQ ID NO:71.

Yet another preferred TCR Vβ of the present invention includes a protein encoded by a nucleic acid molecule which has greater than about 200 base pairs which is preferably about 70% identical, more preferably about 75% identical, more preferably about 80% identical, more preferably about 85% identical, more preferably about 90% identical and even more preferably about 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ

ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73 or SEQ ID NO:74.

The minimal size of such a nucleic acid molecule is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if the nucleic acid molecule is AT-rich. The minimal size of a nucleic acid molecule used to encode a TCR Vβ protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a TCR Vβ protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule encoding a TCR Vβ protein or protein homolog because a nucleic acid molecule of the present invention can include a portion of a gene, an entire gene, or multiple genes. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired. As used herein, "fragments thereof" and "portions thereof" are intended to be used interchangeably, and have a minimal size as disclosed herein.

The minimal size of a protein or nucleic acid molecule of the present invention also can include a portion of a protein or nucleic acid molecule that is less than 100% identical to another protein or nucleic acid molecule, when determined using hybridization or computer alignment methods disclosed herein. For example, a fragment of a hcVβ3 protein of the present invention is at least about 15 residues, preferably 20 residues and more preferably 25 residues in length; a fragment of a hcVβ4 protein of the present invention is at least about 10 residues, preferably 15 residues and more preferably 20 residues in length; a fragment of a hcVβ12 protein of the present invention is at least about 11 residues, preferably 15 residues and more preferably 20 residues in length; a fragment of a hcVβ72 protein of the present invention is at least about 18 residues, preferably 25 residues and more preferably 30 residues in length; or a fragment of a hcVβ21 protein of the present invention is at least about 13 residues, preferably 20 residues and more preferably 25 residues in length. In addition, a nucleic acid molecule fragment of a hcVβ3 nucleic acid molecule of the present invention is at least about 33 nucleotides, preferably 35 nucleotides and more preferably 40 nucleotides in length; a

fragment of a hcVβ4 nucleic acid molecule of the present invention is at least about 21 nucleotides, preferably 25 nucleotides and more preferably 30 nucleotides in length; a fragment of a hcVβ12 nucleic acid molecule of the present invention is at least about 19 nucleotides, preferably 25 nucleotides and more preferably 30 nucleotides in length; a fragment of a hcVβ72 nucleic acid molecule of the present invention is at least about 27 nucleotides, preferably 30 nucleotides and more preferably 35 nucleotides in length; or a fragment of a hcVβ21 nucleic acid molecule of the present invention is at least about 176 nucleotides, preferably 180 nucleotides and more preferably 185 nucleotides in length.

Suitable protein fragments of the present invention include functional portions of a TCR Vβ protein of the present invention including, but not limited to, epitopes, MHC and/or peptide recognition sequences, antigen recognition sequences, superantigen recognition sequences, framework V regions and hypervariable V regions. Preferred functional portions of a TCR Vβ protein include the V, D or J regions. More preferred functional portions of a TCR Vβ protein include: the putative signal peptide encoded by about nucleotide 1 to nucleotide 51, the V region encoded by about nucleotide 52 to about nucleotide 333, and the D/J region encoded by about nucleotide 334 to about nucleotide 381 of SEQ ID NO:1; the putative signal peptide encoded by nucleotide 25 to nucleotide 69, the V region encoded by nucleotide 70 to about nucleotide 351, and the D/J region encoded by about nucleotide 352 to about nucleotide 408 of SEQ ID NO:4; the putative signal peptide encoded by nucleotide 7 to nucleotide 63, the V region encoded by nucleotide 64 to about nucleotide 339, and the D/J region encoded by about nucleotide 340 to about nucleotide 408 of SEQ ID NO:9; the putative signal peptide encoded by nucleotide 85 to nucleotide 141, the V region encoded by nucleotide 142 to about nucleotide 423, and the D/J region encoded by about nucleotide 424 to about nucleotide 438 of SEQ ID NO:98; the putative signal peptide encoded by nucleotide 73 to nucleotide 114, the V region encoded by nucleotide 115 to about nucleotide 396, and the D/J region encoded by about nucleotide 397 to about nucleotide 462 of SEQ ID NO:19; the putative signal peptide encoded by nucleotide 13 to nucleotide 69, the V region encoded by nucleotide 70 to about nucleotide 354, and the D/J region encoded by about nucleotide 355 to about nucleotide 417 of SEQ ID NO:22; and the putative signal

peptide encoded by nucleotide 40 to nucleotide 96, the V region encoded by nucleotide 97 to about nucleotide 369, and the D/J region encoded by about nucleotide 370 to about nucleotide 423 of SEQ ID NO:25.

It is known to those of skill in the art that the junction between the V and D region can vary but that typically the carboxyl end of the V region contains one or more of the amino acid residues alanine or serine following a cysteine. For example, one of skill in the art would know that a conserved carboxyl V region sequence comprises the amino acids CASS. Thus, a V region of the present invention can include the amino acid sequence SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80. The present invention includes nucleic acid molecules having nucleic acid sequences encoding such proteins, which can be identified using the nucleic acid sequences disclosed herein and standard codon usage known to those of skill in the art; such as disclosed, for example, in Lehninger, *Biochemistry*, Worth Publishers, Inc., 1975, which is incorporated herein by this reference in its entirety. For example, a skilled artisan would know that codons encoding serine include ACA, ACC, AGT, AGC or ACG, and codons that encode alanine include GCA, GCC, GCT or GCG.

One embodiment of a TCR Vβ protein of the present invention is a fusion protein that includes a TCR Vβ protein-containing domain attached to one or more fusion segments. It is to be noted that the term "a fusion protein" refers to one or more or at least one fusion protein. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; deliver a TCR Vβ protein or portion thereof to a desired target; act as an immunopotentiator to enhance an immune response against a TCR Vβ protein; and/or assist in purification of a TCR Vβ protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the TCR Vβ-containing domain of the protein and can be susceptible to

cleavage in order to enable straight-forward recovery of a TCR Vβ protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a TCR Vβ -containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of -galactosidase, a strep tag peptide, a T7 tag peptide, a Flag™ peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide. A preferred fusion protein of the present invention includes a TCR Vβ protein of the present invention linked to a TCR alpha chain in such a manner that the beta chain and alpha chain fold correctly to form a functional dimer. Another preferred fusion protein includes a TCR Vβ protein of the present invention linked to at least a portion of the constant region of an immunoglobulin in such a manner that crystallization of the V beta protein is enhanced by the presence of the immunoglobulin sequence.

Preferably a TCR Vβ protein of the present invention is isolated (including isolation of the natural protein or production of the protein by recombinant or synthetic techniques) from canids.

A preferred isolated protein of the present invention is an isolated protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45, SEQ ID NO:48, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including

SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.

A preferred isolated protein of the present invention is a protein encoded by at least one of the following nucleic acid molecules:nCaVβ3$_{381}$, nCaVβ3$_{333}$, nCaVβ3$_{330}$, nCaVβ4$_{408}$, nCaVβ4$_{384}$, nCaVβ4$_{351}$, nCaVβ4$_{339}$, nCaVβ12$_{408}$, nCaVβ12$_{402}$, nCaVβ12$_{339}$, nCaVβ12$_{345}$, nCaVβ72$_{438}$, nCaVβ72$_{399}$, nCaVβ72$_{423}$, nCaVβ72$_{342}$, nCaVβ21$_{462}$, nCaVβ21$_{390}$, nCaVβ21$_{396}$, nCaVβ21$_{348}$, nCaVβ54$_{417}$, nCaVβ54$_{405}$,nCaVβ54$_{354}$, nCaVβ54$_{348}$, nCaVβ182$_{423}$, nCaVβ182$_{384}$, nCaVβ182$_{369}$ and/or nCaVβ182$_{327}$; fragments thereof; or allelic variants of any of these nucleic acid molecules. Another preferred isolated protein is encoded by a nucleic acid molecule having nucleic acid sequence SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80; fragments thereof or an allelic variant of such a nucleic acid molecule.

Translation of SEQ ID NO:1, the coding strand of nCaVβ3$_{381}$, yields a TCR Vβ protein of about 127 amino acids containing the beta chain V, D, and J regions, denoted herein as PCaVβ3$_{127}$, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having a first codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:1 and a last codon spanning from nucleotide 379 through nucleotide 381 of SEQ ID NO:1. The partial putative signal sequence extends from nucleotide 1 to nucleotide 51 of SEQ ID NO:1. The proposed mature protein (i.e.,

canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as $PCaV\beta3_{110}$, contains about 110 amino acids, extending from residue 18 through residue 127 of SEQ ID NO:2. The nucleic acid molecule encoding $PCaV\beta3_{110}$ is denoted herein as $nCaV\beta3_{330}$, extending from nucleotide 52 through nucleotide 381 of SEQ ID NO:1.

Translation of SEQ ID NO:4, the coding strand of $nCaV\beta4_{408}$, yields a protein of about 128 amino acids containing the beta chain V, D, and J regions, denoted herein as $PCaV\beta4_{128}$, the amino acid sequence of which is presented in SEQ ID NO:5, assuming an open reading frame having an initiation codon spanning from nucleotide 25 through nucleotide 27 of SEQ ID NO:4 and a last codon spanning from nucleotide 406 through nucleotide 408 of SEQ ID NO:4. The coding region encoding $PCaV\beta4_{128}$ is presented herein as $nCaV\beta4_{384}$, which has the nucleotide sequence SEQ ID NO:7 (the coding strand) and SEQ ID NO:8 (the complementary strand). The putative signal sequence extends from nucleotide 25 to nucleotide 69 of SEQ ID NO:4. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as $PCaV\beta4_{113}$, contains about 113 amino acids, extending from residue 60 through residue 128 of SEQ ID NO:5. The nucleic acid molecule encoding $PCaV\beta4_{113}$ is denoted herein as $nCaV\beta4_{339}$, extending from nucleotide 70 through nucleotide 408 of SEQ ID NO:4.

Translation of SEQ ID NO:9, the coding strand of $nCaV\beta12_{408}$, yields a protein of about 134 amino acids containing the beta chain V, D, and J regions, denoted herein as $PCaV\beta12_{134}$, the amino acid sequence of which is presented in SEQ ID NO:10, assuming an open reading frame having an initiation codon spanning from nucleotide 7 through nucleotide 9 of SEQ ID NO:9 and a last codon spanning from nucleotide 406 through nucleotide 408 of SEQ ID NO:9. The coding region encoding $PCaV\beta12_{134}$ is presented herein as $nCaV\beta12_{402}$, which has the nucleotide sequence SEQ ID NO:12 (the coding strand) and SEQ ID NO:13 (the complementary strand). The putative signal sequence extends from nucleotide 7 to nucleotide 63 of SEQ ID NO:9. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as $PCaV\beta12_{115}$, contains about 115 amino acids, extending from residue 20 through residue 134 of SEQ ID NO:10. The nucleic acid molecule

encoding PCaVβ12$_{115}$ is denoted herein as nCaVβ12$_{345}$, extending from nucleotide 64 through nucleotide 408 of SEQ ID NO:9.

Translation of SEQ ID NO:98, the coding strand of nCaVβ72$_{438}$, yields a protein of about 133 amino acids containing the beta chain V, D, and J regions, denoted herein as PCaVβ72$_{133}$, the amino acid sequence of which is presented in SEQ ID NO:15, assuming an open reading frame having an initiation codon spanning from nucleotide 85 through nucleotide 87 of SEQ ID NO:98 and a last codon spanning from nucleotide 481 through nucleotide 438 of SEQ ID NO:98. The coding region encoding PCaVβ72$_{133}$ is presented herein as nCaVβ72$_{399}$, which has the nucleotide sequence SEQ ID NO:17 (the coding strand) and SEQ ID NO:18 (the complementary strand). The putative signal sequence extends from nucleotide 85 to nucleotide 141 of SEQ ID NO:98. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ72$_{114}$, contains about 114 amino acids, extending from residue 20 through residue 133 of SEQ ID NO:98. The nucleic acid molecule encoding PCaVβ72$_{114}$ is denoted herein as nCaVβ72$_{342}$, extending from nucleotide 142 through nucleotide 438 of SEQ ID NO:19.

Translation of SEQ ID NO:19, the coding strand of nCaVβ21$_{462}$, yields a protein of about 130 amino acids containing the beta chain V, D, and J regions, denoted herein as PCaVβ21$_{130}$, the amino acid sequence of which is presented in SEQ ID NO:20, assuming an open reading frame having an initiation codon spanning from nucleotide 73 through nucleotide 75 of SEQ ID NO:19 and a last codon spanning from nucleotide 460 through nucleotide 462 of SEQ ID NO:19. The coding region encoding PCaVβ21$_{130}$ is presented herein as nCaVβ21$_{390}$, which extends from nucleotide 73 to nucleotide 462 of SEQ ID NO:19. The putative signal sequence extends from nucleotide 73 to nucleotide 114 of SEQ ID NO:19. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ21$_{116}$, contains about 116 amino acids, extending from residue 15 through residue 130 of SEQ ID NO:19. The nucleic acid molecule encoding PCaVβ21$_{116}$ is denoted herein as nCaVβ21$_{348}$, extending from nucleotide 115 through nucleotide 462 of SEQ ID NO:19.

Translation of SEQ ID NO:22, the coding strand of nCaVβ54$_{417}$, yields a protein of about 135 amino acids containing the beta chain V, D, and J regions, denoted herein

as PCaVβ54_{135}, the amino acid sequence of which is presented in SEQ ID NO:23, assuming an open reading frame having an initiation codon spanning from nucleotide 13 through nucleotide 15 of SEQ ID NO:22 and a last codon spanning from nucleotide 415 through nucleotide 417 of SEQ ID NO:22. The coding region encoding PCaVβ54_{135} is presented herein as nCaVβ54_{405}, which extends from nucleotide 13 to nucleotide 417 of SEQ ID NO:22. The putative signal sequence extends from nucleotide 13 to nucleotide 69 of SEQ ID NO:22. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ54_{116}, contains about 116 amino acids, extending from residue 20 through residue 135 of SEQ ID NO:22. The nucleic acid molecule encoding PCaVβ54_{116} is denoted herein as nCaVβ54_{348}, extending from nucleotide 70 through nucleotide 417 of SEQ ID NO:22.

Translation of SEQ ID NO:25, the coding strand of nCaVβ182_{423}, yields a protein of about 128 amino acids containing the beta chain V, D, and J regions, denoted herein as PCaVβ182_{128}, the amino acid sequence of which is presented in SEQ ID NO:26, assuming an open reading frame having an initiation codon spanning from nucleotide 40 through nucleotide 43 of SEQ ID NO:25 and a last codon spanning from nucleotide 421 through nucleotide 423 of SEQ ID NO:25. The coding region encoding PCaVβ182_{128} is presented herein as nCaVβ182_{384}, which extends from nucleotide 40 to nucleotide 423 of SEQ ID NO:25. The putative signal sequence extends from nucleotide 40 to nucleotide 96 of SEQ ID NO:25. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ182_{109}, contains about 109 amino acids, extending from residue 20 through residue 128 of SEQ ID NO:25. The nucleic acid molecule encoding PCaVβ182_{109} is denoted herein as nCaVβ182_{327}, extending from nucleotide 97 through nucleotide 423 of SEQ ID NO:25.

Translation of SEQ ID NO:28, the coding strand of nCaVβ3_{333}, yields a protein of about 111 amino acids containing the beta chain V region, denoted herein as PCaVβ3_{111}, the amino acid sequence of which is presented in SEQ ID NO:29, assuming an open reading frame having a first codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:28 and a last codon spanning from nucleotide 331 through

nucleotide 333 of SEQ ID NO:28. The complement of SEQ ID NO:28 is SEQ ID NO:30.

Translation of SEQ ID NO:31, the coding strand of nCaV$\beta 4_{351}$, yields a protein of about 109 amino acids containing the beta chain V region, denoted herein as PCaV$\beta 4_{109}$, the amino acid sequence of which is presented in SEQ ID NO:32, assuming an open reading frame having a first codon spanning from nucleotide 25 through nucleotide 27 of SEQ ID NO:31 and a last codon spanning from nucleotide 349 through nucleotide 351 of SEQ ID NO:31. The complement of SEQ ID NO:31 is SEQ ID NO:33.

Translation of SEQ ID NO:34, the coding strand of nCaV$\beta 12_{339}$, yields a protein of about 111 amino acids containing the beta chain V region, denoted herein as PCaV$\beta 12_{111}$, the amino acid sequence of which is presented in SEQ ID NO:35, assuming an open reading frame having a first codon spanning from nucleotide 7 through nucleotide 9 of SEQ ID NO:34 and a last codon spanning from nucleotide 337 through nucleotide 339 of SEQ ID NO:34. The complement of SEQ ID NO:34 is SEQ ID NO:35.

Translation of SEQ ID NO:37, the coding strand of nCaV$\beta 72_{423}$, yields a protein of about 113 amino acids containing the beta chain V region, denoted herein as PCaV$\beta 72_{113}$, the amino acid sequence of which is presented in SEQ ID NO:38, assuming an open reading frame having a first codon spanning from nucleotide 85 through nucleotide 87 of SEQ ID NO:37 and a last codon spanning from nucleotide 421 through nucleotide 423 of SEQ ID NO:37. The complement of SEQ ID NO:37 is SEQ ID NO:39.

Translation of SEQ ID NO:40, the coding strand of nCaV$\beta 21_{396}$, yields a protein of about 108 amino acids containing the beta chain V region, denoted herein as PCaV$\beta 21_{108}$, the amino acid sequence of which is presented in SEQ ID NO:41, assuming an open reading frame having a first codon spanning from nucleotide 73 through nucleotide 75 of SEQ ID NO:40 and a last codon spanning from nucleotide 394 through nucleotide 396 of SEQ ID NO:40. The complement of SEQ ID NO:40 is SEQ ID NO:42.

Translation of SEQ ID NO:43, the coding strand of nCaVβ54$_{354}$, yields a protein of about 114 amino acids containing the beta chain V region, denoted herein as PCaVβ54$_{114}$, the amino acid sequence of which is presented in SEQ ID NO:44, assuming an open reading frame having a first codon spanning from nucleotide 13 through nucleotide 15 of SEQ ID NO:43 and a last codon spanning from nucleotide 352 through nucleotide 354 of SEQ ID NO:43. The complement of SEQ ID NO:43 is SEQ ID NO:45. Translation of SEQ ID NO:46, the coding strand of nCaVβ182$_{369}$, yields a protein of about 110 amino acids containing the beta chain V region, denoted herein as PCaVβ182$_{110}$, the amino acid sequence of which is presented in SEQ ID NO:47, assuming an open reading frame having a first codon spanning from nucleotide 40 through nucleotide 42 of SEQ ID NO:46 and a last codon spanning from nucleotide 367 through nucleotide 369 of SEQ ID NO:46. The complement of SEQ ID NO:46 is SEQ ID NO:48.

Preferred TCR Vβ proteins of the present invention include proteins that are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to PCaVβ3$_{127}$; are at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to PCaVβ4$_{128}$; are at least about 57%, preferably at least about 60%, even more preferably at least about 65%, and even more preferably at least about 70% identical to PCaVβ12$_{134}$; are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to PCaVβ72$_{133}$; or are at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to PCaVβ21$_{130}$. More preferred are TCR Vβ proteins comprising PCaVβ3$_{127}$, PCaVβ3$_{111}$, PCaVβ3$_{110}$, PCaVβ4$_{128}$, PCaVβ4$_{113}$, PCaVβ4$_{109}$, PCaVβ12$_{134}$, PCaVβ12$_{111}$, PCaVβ12$_{115}$, PCaVβ72$_{133}$, PCaVβ72$_{113}$, PCaVβ72$_{114}$, PCaVβ21$_{130}$, PCaVβ21$_{108}$, PCaVβ21$_{116}$, PCaVβ54$_{135}$, PCaVβ54$_{114}$, PCaVβ54$_{116}$, PCaVβ182$_{128}$, PCaVβ182$_{110}$, PCaVβ182$_{109}$ and fragments thereof; and proteins encoded by allelic variants of nucleic acid molecules encoding proteins PCaVβ3$_{127}$, PCaVβ3$_{111}$, PCaVβ3$_{110}$, PCaVβ4$_{128}$, PCaVβ4$_{113}$, PCaVβ4$_{109}$, PCaVβ12$_{134}$, PCaVβ12$_{111}$, PCaVβ12$_{115}$, PCaVβ72$_{133}$, PCaVβ72$_{113}$, PCaVβ72$_{114}$, PCaVβ21$_{130}$, PCaVβ21$_{108}$, PCaVβ21$_{116}$,

PCaVβ54_{135}, PCaVβ54_{114}, PCaVβ54_{116}, PCaVβ182_{128}, PCaVβ182_{110} and/or PCaVβ182_{109},and fragments thereof.

Other preferred TCR Vβ proteins of the present invention include proteins having amino acid sequences that are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to SEQ ID NO:2; at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to SEQ ID NO:5; are at least about 57%, preferably at least about 60%, even more preferably at least about 65%, and even more preferably at least about 70% identical to SEQ ID NO:10; are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to SEQ ID NO:15; or are at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to SEQ ID NO:20. More preferred are TCR Vβ proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80 and/or proteins encoded by the complement of a nucleic acid sequence including SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56; and TCR Vβ proteins encoded by allelic variants of nucleic acid molecules encoding TCR Vβ proteins having amino acid sequences SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID

NO:78, SEQ ID NO:79, SEQ ID NO:80 and/or proteins encoded by the complement of a nucleic acid sequence including SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56; and fragments of any of such amino acid sequences.

A preferred isolated protein of the present invention comprises a protein selected from the group consisting of:(a) an isolated protein having an amino acid sequence that is at least about 55 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 amino acids in length; (b) an isolated protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 15 amino acids in length; (c) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:35, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 25 amino acids in length; and (d) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:38, SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 35 amino acids in length.

A preferred isolated protein of the present invention comprises a protein selected from the group consisting of:(a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length; (b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment that is

at least about 30 nucleotides in length; (c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and (d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

It is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCG™ (available from Genetics Computer Group, Madison, WI), DNAsis™ (available from Hitachi Software, San Bruno, CA) and MacVector™ (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Compare function by maximum matching within the program DNAsis Version 2.1.

Additional preferred TCR Vβ proteins of the present invention include proteins encoded by nucleic acid molecules comprising at least a portion of $nCaV\beta3_{381}$, $nCaV\beta3_{333}$, $nCaV\beta3_{330}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta4_{351}$, $nCaV\beta4_{339}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta12_{339}$, $nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$,$nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ and/or

nCaVβ182$_{327}$, fragments thereof, as well as TCR Vβ proteins encoded by allelic variants of such nucleic acid molecules.

Also preferred are TCR Vβ proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, fragments thereof, as well as allelic variants of these nucleic acid molecules.

The present invention also includes mimetopes of TCR Vβ proteins of the present invention. As used herein, a mimetope of a TCR Vβ protein of the present invention refers to any compound that is able to mimic the activity of a TCR Vβ protein of the present invention, often because the mimetope has a structure that mimics the particular TCR Vβ protein. It is to be noted that the term "a mimetope" refers to one or more or at least one mimetope. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, RNA or other organic molecules, non-organic molecules and screening such samples by affinity chromatography techniques using the corresponding binding partner.

Another embodiment of the present invention includes a TCR Vβ nucleic acid molecule. It is to be noted that the term "a nucleic acid molecule homolog" refers to one or more or at least one nucleic acid molecule homologs. In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated TCR Vβ nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated TCR Vβ nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode a TCR Vβ protein of the present invention.

A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid with the complementary sequence of another nucleic acid molecule. As such, the minimal size of a TCR Vβ nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

A TCR Vβ nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, *ibid.*. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a TCR Vβ nucleic acid molecule or by screening the function of a protein encoded

by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a TCR Vβ protein).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one TCR Vβ protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a TCR Vβ protein.

A preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of regulating an immune response in an animal. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode an immunoregulatory protein (e.g., a cell-bound or soluble TCR Vβ protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e, as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine.

One embodiment of the present invention is a TCR Vβ nucleic acid molecule comprising all or part of nucleic acid molecules $nCaV\beta3_{381}$, $nCaV\beta3_{333}$, $nCaV\beta3_{330}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta4_{351}$, $nCaV\beta4_{339}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta12_{339}$, $nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$,$nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ and/or $nCaV\beta182_{327}$, or allelic variants of these nucleic acid molecules. Another preferred nucleic acid molecule comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27,SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID

NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, and complements thereof; as well as fragments thereof; as well as allelic variants of nucleic acid molecules having these nucleic acid sequences. Such nucleic acid molecules can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, a full-length gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent therapeutic compound.

In one embodiment, a TCR Vβ nucleic acid molecule of the present invention encodes a protein that is at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to PCaVβ3_{127}; are at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to PCaVβ4_{128}; are at least about 57%, preferably at least about 60%, even more preferably at least about 65%, and even more preferably at least about 70% identical to PCaVβ12_{134}; are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to PCaVβ72_{133}; or are at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to PCaVβ21_{130}. Even more preferred is a nucleic acid molecule encoding PCaVβ3_{127}, PCaVβ3_{111}, PCaVβ3_{110}, PCaVβ4_{128}, PCaVβ4_{113}, PCaVβ4_{109}, PCaVβ12_{134}, PCaVβ12_{111}, PCaVβ12_{115}, PCaVβ72_{133}, PCaVβ72_{113}, PCaVβ72_{114}, PCaVβ21_{130}, PCaVβ21_{108}, PCaVβ21_{116}, PCaVβ54_{135},

PCaVβ54$_{114}$, PCaVβ54$_{116}$, PCaVβ182$_{128}$, PCaVβ182$_{110}$ and/or PCaVβ182$_{109}$, fragments thereof, complements thereof, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, a TCR Vβ nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to SEQ ID NO:2; are at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to SEQ ID NO:5; are at least about 57%, preferably at least about 60%, even more preferably at least about 65%, and even more preferably at least about 70% identical to SEQ ID NO:10; are at least about 65%, preferably at least about 70%, even more preferably at least about 75%, and even more preferably at least about 80% identical to SEQ ID NO:15; or at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to SEQ ID NO:20. The present invention also includes a TCR Vβ nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80 and/or proteins encoded by the complement of a nucleic acid sequence including SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56; fragments thereof; complements thereof, as well as allelic variants of a TCR Vβ nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a TCR Vβ nucleic acid molecule of the present invention is at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to nCaVβ3$_{381}$; is at least

about 75%, preferably at least about 80%, even more preferably at least about 85%, and even more preferably at least about 90% identical to nCaVβ4$_{408}$; is at least about 72%, preferably at least about 80%, even more preferably at least about 85%, and even more preferably at least about 90% identical to nCaVβ12$_{408}$; is at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to nCaVβ72$_{438}$; or is at least about 38%, preferably at least about 45%, even more preferably at least about 50%, and even more preferably at least about 55% identical to nCaVβ21$_{462}$. Even more preferred is a nucleic acid molecule comprising nCaVβ3$_{381}$, nCaVβ3$_{333}$, nCaVβ3$_{330}$, nCaVβ4$_{408}$, nCaVβ4$_{384}$, nCaVβ4$_{351}$, nCaVβ4$_{339}$, nCaVβ12$_{408}$, nCaVβ12$_{402}$, nCaVβ12$_{339}$, nCaVβ12$_{345}$, nCaVβ72$_{438}$, nCaVβ72$_{399}$, nCaVβ72$_{423}$, nCaVβ72$_{342}$, nCaVβ21$_{462}$, nCaVβ21$_{390}$, nCaVβ21$_{396}$, nCaVβ21$_{348}$, nCaVβ54$_{417}$, nCaVβ54$_{405}$,nCaVβ54$_{354}$, nCaVβ54$_{348}$, nCaVβ182$_{423}$, nCaVβ182$_{384}$, nCaVβ182$_{369}$ and/or nCaVβ182$_{327}$; fragments thereof; complements thereof; as well as an allelic variant of such a nucleic acid molecule.

In another embodiment, a TCR Vβ nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 69%, preferably at least about 75%, even more preferably at least about 80%, and even more preferably at least about 85% identical to SEQ ID NO:1; is at least about 75%, preferably at least about 80%, even more preferably at least about 85%, and even more preferably at least about 90% identical to SEQ ID NO:4; is at least about 72%, preferably at least about 80%, even more preferably at least about 85%, and even more preferably at least about 90% identical to SEQ ID NO:9; is at least about 60%, preferably at least about 65%, even more preferably at least about 70%, and even more preferably at least about 75% identical to SEQ ID NO:98; or is at least about 38%, preferably at least about 45%, even more preferably at least about 50%, and even more preferably at least about 55% identical to SEQ ID NO:19. The present invention also includes a TCR Vβ nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27,SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID

NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, and complements thereof; fragments thereof; complements thereof; as well as allelic variants of such TCR Vβ nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

A preferred isolated nucleic acid molecule of the present invention comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:100, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45, SEQ ID NO:48, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or the complement of a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80; or fragments thereof.

A preferred isolated nucleic acid molecule of the present invention comprises a nucleic acid sequence that is any of the following: (a) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group

consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:30 and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length; (b) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:33, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length; (c) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, SEQ ID NO:36, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:67, or a fragment thereof that is at least about 40 nucleotides in length; (d) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:39, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length; and (e) a nucleic acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

A preferred isolated nucleic acid molecule of the present invention comprises a nucleic acid sequence encoding a protein selected from the group consisting of: (a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length; (b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length; (c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and (d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30, or a fragment thereof, wherein said fragment has an at least a 20 contiguous nucleotide region identical in sequence to a 20 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30; (b) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the

group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, and SEQ ID NO:33, or a fragment thereof, wherein said fragment has an at least a 25 contiguous nucleotide region identical in sequence to a 25 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31 and SEQ ID NO:33; (c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36, or a fragment thereof, wherein said fragment has an at least a 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36; and (d) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39, or a fragment thereof, wherein said fragment has an at least a 60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39. The phrase, a homolog having an at least "x" contiguous nucleotide region identical in sequence to an "x" contiguous nucleotide region of a nucleic acid molecule selected from the group consisting of SEQ ID NO:"y", refers to an "x"-nucleotide in length nucleic acid molecule that is identical in sequence to an "x"-nucleotide portion of SEQ ID NO:"y", as well as to nucleic acid molecules that are longer in length than "x". The additional length may be in the form of nucleotides that extend from either the 5' or the 3' end(s) of the contiguous identical "x"-nucleotide portion. The 5' and/or 3' extensions can include one or more extensions that have no identity to an immunoregulatory molecule of the present invention, as well as extensions that show similarity or identity to cited nucleic acids sequences or portions thereof.

Knowing the nucleic acid sequences of certain TCR Vβ nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a

portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other TCR Vβ nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include mammalian cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include mammalian cDNA and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising TCR Vβ nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. A preferred oligonucleotide of the present invention has a minimum size of about 12 nucleotides. Preferably, an oligonucleotide of the present invention has a size from about 12 nucleotides to about 30 nucleotides and more preferably from about 15 nucleotides to about 25 nucleotides.

A preferred isolated oligonucleotide of the present invention comprises a unique nucleic acid sequence within a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27,SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID

NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, and complements thereof; and a homolog thereof.

The present invention includes oligonucleotides that can be used, for example, as probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit TCR Vβ protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

Preferred oligonucleotides of the present invention include oligonucleotides comprising a unique nucleic acid sequence, as defined herein, within a nucleic acid molecule of the present invention or homologs thereof. Preferred homologs of an oligonucleotide are capable of priming a nucleic acid sequence.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the

cloning, sequencing, and/or otherwise manipulating of TCR Vβ nucleic acid molecules of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, insect and mammalian cells, and more preferably in the cell types disclosed herein

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other endoparasite, or insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and

fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with mammals, such as canine or feline transcription control sequences.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include $nCaV\beta3_{381}$, $nCaV\beta3_{333}$, $nCaV\beta3_{330}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta4_{351}$, $nCaV\beta4_{339}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta12_{339}$, $nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$, $nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ and/or $nCaV\beta182_{327}$.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed TCR Vβ protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a

nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include TCR Vβ nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include $nCaV\beta3_{381}$, $nCaV\beta3_{333}$, $nCaV\beta3_{330}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta4_{351}$, $nCaV\beta4_{339}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta12_{339}$, $nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$, $nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ and/or $nCaV\beta182_{327}$.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing TCR Vβ proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells

of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), other insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, helminth, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Pichia*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, chinese hamster ovary (CHO) cells, Ltk cells and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1 $_{0}$3987 and SR-11 $_{0}$4072; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK^{31} cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein.

It is to be noted that the term "a recombinant molecule", "a host cell" or "a recombinant cell" refers to one or more or at least one recombinant molecule, host cell or recombinant cell, respectively.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including TCR Vβ nucleic acid molecules encoding one or more proteins of the present invention and one or more other nucleic acid molecules encoding other therapeutic compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated TCR Vβ proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective, medium refers to any medium in which a cell is cultured to produce a TCR Vβ protein

of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a TCR Vβ protein of the present invention or a mimetope thereof (e.g., anti-TCR Vβ antibodies). As used herein, the term "selectively binds to" a TCR Vβ protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including

enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.* An anti- TCR Vβ antibody of the present invention preferably selectively binds to a TCR Vβ protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce TCR Vβ proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as reagents in assays to detect TCR Vβ protein, (b) as reagents in assays to modulate cellular activity through a TCR Vβ protein (e.g., mimicking ligand binding to TCR Vβ protein), and/or (c) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target compounds (e.g., nucleic acid molecules, drugs or proteins) to antigen presenting cells. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the compounds using techniques known to those skilled in the art. Suitable compounds are known to those skilled in the art.

One embodiment of the present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of regulating an immune response in an animal. Therapeutic compositions of the present invention include at least one of the following therapeutic compounds: an isolated TCR Vβ protein of the present invention or a mimetope thereof, an isolated TCR Vβ nucleic acid molecule of the present invention, an isolated antibody that selectively binds to a TCR Vβ protein of the present invention, an inhibitor of TCR Vβ function identified by its ability to bind to a TCR Vβ protein of the present invention and inhibit binding of a TCR Vβ protein to MHC, and a mixture thereof (i.e., combination of at least two of the compounds). As used herein, a therapeutic compound refers to a compound that, when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent a disease. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

The present invention also includes a therapeutic composition comprising at least one TCR Vβ-based compound of the present invention in combination with at least one additional therapeutic compound. Examples of such compounds are disclosed herein.

Therapeutic compositions of the present invention can be administered to any animal susceptible to such therapy, preferably to mammals, and more preferably to dogs.

A therapeutic composition of the present invention is administered to an animal in an effective manner such that the composition is capable of regulating an immune response in that animal. Therapeutic compositions of the present invention can be administered to animals prior to onset of a disease (i.e., as a preventative vaccine) and/or can be administered to animals after onset of a disease in order to treat the disease (i.e., as a therapeutic vaccine). Preferred diseases to prevent or treat include autoimmune diseases, allergic reactions, infectious diseases and cancer.

Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include

suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., Flt-3 ligand, granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein. A therapeutic composition can contain one or more adjuvants.

In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols. A therapeutic composition can contain one or more carriers.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition to regulate an immune response in an animal. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

Therapeutic compositions of the present invention can be administered to animals prior to and/or after onset of disease. Acceptable protocols to administer therapeutic compositions in an effective manner include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of regulating the immune response in an animal when administered

one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody therapeutic composition is from about 1 microgram (g) to about 10 milligrams (mg) of the therapeutic composition per kilogram body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 g to about 1 mg of the therapeutic composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, subcutaneous, intradermal, intravenous, intranasal, intraoccular, oral, transdermal and intramuscular routes.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a therapeutic protein or therapeutic RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked (i.e., not packaged in a viral coat or cellular membrane) nucleic acid as a genetic vaccine (e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle). One or more nucleic acid molecules can be delivered to an animal.

A genetic (i.e., naked nucleic acid) vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a portion of a viral genome (i.e., a viral vector). Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses,

with those based on alphaviruses (such as sindbis or Semliki forest virus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 g, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized and/or topically. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (such as Sindbis virus), raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a therapeutic protein or RNA nucleic acid molecule that is capable of protecting the

animal from disease caused by a parasitic helminth as disclosed herein. For example, a recombinant virus vaccine comprising a TCR Vβ nucleic acid molecule of the present invention is administered according to a protocol that results in the regulation of an immune response in an animal. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1×10^4 to about 1×10^8 virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal, intraoccular and oral administration routes being preferred. One or more recombinant virus vaccines can be delivered to an animal.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda*, yeast, (including *Saccharomyces cerevisiae and Pichia pastoris*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a therapeutic composition of the present invention to regulate the immune response in an animal can be tested in a variety of ways including, but not limited to, detection of cellular immunity within the treated animal, determination of T cell activity (helper or cytotoxic T cell activity), identification of T cell repertoire, detection of immunoregulatory cytokines, e.g., IL-2, IL-4, IL-10, IL-12, levels, detection of antibody levels, determine tumor development or challenge of the treated animal with an infectious agent to determine whether the treated animal is resistant to disease. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

According to the present invention, a therapeutic composition is used to treat a disease requiring immunological regulation, such as cancer, infectious diseases,

autoimmune diseases or allergy. Suitable cancers to treat include lymphomas or leukemias. Suitable infectious diseases to treat include diseases caused by viral, bacterial, yeast, fungal or parasitic infection. Suitable autoimmune diseases to treat include: autoimmune skin diseases, e.g. pemphigus foliaceous, pemphigus vulgaris, pemphigus vegetans, pemphigus erythematosus, bollous pemphigoid, discoid lupus, dermatomyositis or subcorneal pustular dermatosis; blood disorders, e.g. autoimmune hemolytic anemia, immune-mediated thrombocytopenia, aplstic anemia, pure red cell aplasia or immune mediated neutropenia; endocrine dysfuntion, e.g. lymphocytic thyroditis, diabetes, hypoadrenocorticism or hypoparathyroidism; multi-system dysfunction, e.g. systemic lupus erythematosus or Sjogren's syndrome; neurologic dysfunction, e.g. myasthenia gravis, distemper and rabies post vaccinal encephalopathy or acute polyradiculoneuritis; or musculoskeletal disease, e.g. rheumatoid arthritis, idiopathic polyarthritis, plasmacytic-lymphocytic arthritis or polymyosutus. Suitable allergies to treat include allergic dermatitis, atopic dermatitis, allergic rhinitis or allergic bronchitis.

One therapeutic composition of the present invention includes a TCR Vß protein of the present invention, or a portion of such TCR Vß protein that elicits a cytotoxic T cell response against a T cell bearing the TCR Vß protein. A preferred TCR Vß protein comprises a soluble form of a TCR Vß protein of the present invention, with a peptide of a TCR Vß protein being more preferred. A preferred TCR Vß peptide is from about 5 to about 50 residues, more preferably from about 10 to about 40 residues and even more preferably from about 12 to about 30 residue in length. One or more TCR Vß proteins of the present invention, or portions of such TCR Vß proteins can be used in a therapeutic composition.

According to the present invention, a therapeutic composition comprising a TCR Vß peptide, e.g. a portion of a polypeptide, can be delivered to an animal as a peptide or in the form of DNA encoding such peptide. A TCR Vß peptide of the present invention can be linked to another molecule to assist in the delivery of the peptide to an animal. Preferably, a TCR Vß peptide of the present invention is administered systemically to an animal. One or more peptides can be used in a therapeutic composition.

Another therapeutic composition of the present invention includes an inhibitory compound that inhibits a TCR Vß protein from binding to MHC. An inhibitory compound is capable of substantially interfering with the function of a TCR Vß protein susceptible to inhibition. For example, an inhibitory compound is administered in an amount and manner that inhibits an immune response that is sufficient to treat an animal for a disease that requires downregulation of an immune response. One or more inhibitors can be used in a therapeutic composition.

Suitable inhibitory compounds include compounds that prevent the activation of an immunoregulatory cell through TCR Vß by, for example, interfering with the binding of TCR Vß protein to MHC by binding to either the TCR Vß protein or MHC. An example of an inhibitory compound is an antibody of the present invention, administered to an animal in an effective manner; i.e., an antibody of the present invention, is administered in an amount so as to be present in the animal at a titer that is sufficient, upon interaction of that antibody with a native TCR Vß protein, to decrease TCR Vß activity in an animal, at least temporarily. Oligonucleotide nucleic acid molecules of the present invention can also be administered in an effective manner, thereby reducing expression of TCR Vß proteins in order to interfere with TCR Vß activity targeted in accordance with the present invention. Peptides of TCR Vß proteins of the present invention can also be administered in an effective manner, thereby reducing binding of TCR Vß proteins to MHC in order to interfere with TCR Vß activity targeted in accordance with the present invention. Preferably, an inhibitory compound is derived from a TCR Vß protein of the present invention.

An inhibitory compound of TCR Vß function can be identified using TCR Vß proteins of the present invention. One embodiment of the present invention is a method to identify a compound capable of inhibiting TCR Vß function. Such a method includes the steps of: (a) contacting (e.g., combining, mixing) an isolated TCR Vß protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein binds to a protein including MHC, and (b) determining if the putative inhibitory compound inhibits the binding of TCR Vß to MHC. Putative inhibitory compounds to screen include small organic molecules, antibodies (including mimetopes thereof), and ligand analogs. Such compounds can

also be screened to identify those compounds that are substantially not toxic to a recipient animal.

One embodiment of the present invention is a method to detect expansion of T cells in an animal. The present method utilizes the discovery by the inventors of unique sequences in a TCR Vß and more particularly in the V region of a TCR Vß that function, or can be used, as markers for T cells. As used herein, the term "unique sequences" refers to nucleic acid or amino acid sequences that are present in one TCR Vß nucleic acid molecule or TCR Vß protein, but not in another TCR Vß nucleic acid molecule or TCR Vß protein, respectively. Thus, a unique sequence differentiates one TCR Vß nucleic acid molecule or TCR Vß protein of the present invention from another TCR Vß nucleic acid molecule or TCR Vß protein, respectively. For example, a unique sequence within hcVβ3, is not found within hcVβ4, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182, and a unique sequence within hcVβ4, is not found within hcVβ3, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182, and so on. A unique sequence of the present invention can be used to detect expansion of T cells by, for example, determining the presence or absence of any one or more unique sequences, determining increased or decreased levels of molecules carrying one or more unique sequences compared with other unique sequences in the same animal and/or a different animal, or comparing levels of different TCR Vß proteins in an animal.

It is within the scope of the present invention that any unique sequence present in a TCR Vß sequence of the present invention can be used in the practice of the present method, including those identified herein and those which will be identified based on the sequences of nucleic acid molecules or proteins disclosed herein. Preferably, unique sequences of the present invention include those sequences located within about the first 200 nucleotides or about the first 70 residues of the 5' or amino terminus of a nucleic acid molecule or a protein. The suitable length of a unique sequence depends upon the reagent used to detect the presence of the unique sequence. For example, a suitable length of a unique sequence is from about 15 to about 30 nucleotides if the detection reagent is a DNA primer. Alternatively, a suitable length of a unique sequence is from about 50 to about 300 nucleotides if the detection reagent is a DNA hybridization probe. Alternatively, a suitable length of a unique sequence is at least about 5 amino acids if the

detection reagent is an antibody. In addition, one or more unique sequences can be identified, and one or more reagents can be used to identify such sequences, using a method of the present invention.

Any method of the present invention can be used to determine the presence, absence, amount or ratio of TCR Vß's of the present invention in an animal to determine T cell expansion and/or diagnose an abnormal state or a specific disease. Typically, numbers of T cells in an animal are regulated. Expansion of total T cell numbers, or of a particular T cell clone, can represent an abnormal state or disease. For example, a particular T cell clone is expanded in a T cell lymphoma or leukemia. Thus, information derived using the present methods is particularly useful because the inventors have discovered the seven different TCR Vß proteins disclosed herein which can comprise at least about 95% of the TCR Vß repertoire in canids. The discovery of the existence of a small repertoire of TCR Vß proteins enables the production of appropriate reagents that detect a substantial amount of T cell receptors in an animal. The reagents can be used to correlate T cell expansion with a specific disease by comparing results obtained using samples from normal animals compared with animals having or suspected of having a disease. Thus, any diagnostic method of the present invention is useful for determining if an animal is susceptible to, has or is in remission from a disease.

According to the present invention, T cell expansion is determined by detecting increased levels of one or more specific TCR Vß molecules in a tissue sample isolated from an animal. Increased levels of TCR Vß molecules can be determined by comparing levels of two or more different TCR Vß molecules in a tissue sample isolated from one animal, or levels of one or more TCR Vß molecules in tissue samples from two or more different animals. Total amounts or ratios of particular TCR Vß molecules can be determined by one of skill in the art depending upon the method used to detect the presence of a TCR Vß molecule in a sample. Preferably, a ratio illustrative of increased levels of a particular TCR Vß molecule is from about 2-fold to about 5-fold, more preferably from about 5-fold to about 25, and more preferably 10-fold more of one particular TCR Vß molecule compared with another sample of the same TCR Vß molecule or a different TCR Vß molecule. Methods to determine ratios are known to

those in the art and include, for example, densitometry, spectrophotometry or determining counts per minute of isotopes.

One embodiment of the present invention is a method to detect expansion of T cells in an animal comprising detecting the presence of one or more T cell receptors having unique nucleic acid sequences within hcVβ3, hcVβ4, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182 nucleic acid molecules or hcVβ3, hcVβ4, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182 proteins by forming detectable products, wherein the increased level of a detectable product compared with another detectable product indicates expansion of the T cells. According to the present method, a suitable sample containing T cells is isolated from an animal. Samples containing T cell receptors can be isolated from the same or different animals. Control samples can be obtained from the same or different animals. The sample is contacted under appropriate conditions with one or more reagents capable of identifying one or more unique nucleic acid or amino acid sequences, respectively. Preferably, a reagent distinguishes one member of the group comprising hcVβ3, hcVβ4, hcVβ12, hcVβ72, hcVβ21, hcVβ54 or hcVβ182 nucleic acid molecules or hcVβ3, hcVβ4, hcVβ12, hcVβ72, hcVβ21, hcVβ54 or hcVβ182 proteins, preferably $nCaV\beta3_{381}$, $nCaV\beta4_{408}$, $nCaV\beta12_{408}$, $nCaV\beta72_{438}$, $nCaV\beta21_{462}$, $nCaV\beta54_{417}$, $nCaV\beta182_{423}$, $PCaV\beta3_{127}$, $PCaV\beta4_{128}$, $PCaV\beta12_{134}$, $PCaV\beta72_{133}$, $PCaV\beta21_{130}$, $PCaV\beta54_{135}$ or $PCaV\beta182_{128}$, from another member of that group. By determining increased production of one of the detectable products, one can detect T cell expansion. Preferably, expansion of a T cell is determined by comparing formation of one detectable product with formation of one or more other detectable products and looking for increased production of at least one of the products compared to another. According to the present invention, a detectable product can comprise a nucleic acid molecule, a peptide, a protein or an antibody. Preferred detectable products and methods to form detectable products are disclosed herein. It is within the skill of one in the art that methods to identify detectable products based on the product being detected.

The invention also provides novel reagents useful in the methods which have been described herein above. Thus, the invention includes reagents capable of binding to unique nucleic acid or unique amino acid sequences contained within a TCR Vß. Preferred reagents include those which can differentiate one TCR V beta protein, or

nucleic acid molecule, from another. Preferred reagents can distinguish TCR V beta proteins hcVβ2, hcVβ3, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182, or the nucleic acid molecules that encode them, from each other. More preferred reagents can distinguish proteins comprising PCaV$\beta 3_{127}$, PCaV$\beta 3_{111}$, PCaV$\beta 3_{110}$, PCaV$\beta 4_{128}$, PCaV$\beta 4_{113}$, PCaV$\beta 4_{109}$, PCaV$\beta 12_{134}$, PCaV$\beta 12_{111}$, PCaV$\beta 12_{115}$, PCaV$\beta 72_{133}$, PCaV$\beta 72_{113}$, PCaV$\beta 72_{114}$, PCaV$\beta 21_{130}$, PCaV$\beta 21_{108}$, PCaV$\beta 21_{116}$, PCaV$\beta 54_{135}$, PCaV$\beta 54_{114}$, PCaV$\beta 54_{116}$, PCaV$\beta 182_{128}$, PCaV$\beta 182_{110}$ and/or PCaV$\beta 182_{109}$ from each other, or nucleic acid molecules comprising nCaV$\beta 3_{381}$, nCaV$\beta 3_{333}$, nCaV$\beta 3_{330}$, nCaV$\beta 4_{408}$, nCaV$\beta 4_{384}$, nCaV$\beta 4_{351}$, nCaV$\beta 4_{339}$, nCaV$\beta 12_{408}$, nCaV$\beta 12_{402}$, nCaV$\beta 12_{339}$, nCaV$\beta 12_{345}$, nCaV$\beta 72_{438}$, nCaV$\beta 72_{399}$, nCaV$\beta 72_{423}$, nCaV$\beta 72_{342}$, nCaV$\beta 21_{462}$, nCaV$\beta 21_{390}$, nCaV$\beta 21_{396}$, nCaV$\beta 21_{348}$, nCaV$\beta 54_{417}$, nCaV$\beta 54_{405}$,nCaV$\beta 54_{354}$, nCaV$\beta 54_{348}$, nCaV$\beta 182_{423}$, nCaV$\beta 182_{384}$, nCaV$\beta 182_{369}$ and/or nCaV$\beta 182_{327}$ from each other. Even more preferred reagents can distinguish TCR V beta proteins comprising amino acid sequence SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47 from each other.

In particular, a method of the present invention comprises: (a) contacting a sample, i.e., one or more samples, containing DNA from T cells with a reagent, i.e., one or more reagents, having specificity for a unique nucleic acid sequence; and (b) determining the presence of DNA carrying the unique nucleic acid sequences. Methods to determine the presence of the DNA are disclosed herein.

In one embodiment, identifying the presence of a T cell receptor having unique sequence can be achieved by polymerase chain reaction (PCR) amplification techniques known to those of skill in the art. The PCR amplification forms a detectable product comprising DNA. An example of a suitable reagent to use in PCR amplification techniques is a DNA primer complementary to all or a portion of a unique nucleic acid sequence, referred to herein as a unique sequence primer. Preferably, another primer is used in conjunction with the unique sequence primer in order o effect amplification. This second primer can be complementary to a unique sequence or to a common sequence (i.e., a sequence shared by different TCR beta chain sequences). Typically, the second primer is complementary to a common sequence and is chosen based on its distance from the unique sequence primer, i.e. based on ease of detection of PCR

amplification product. One of skill in the art understands that a preferred product of a PCR reaction is from about 100 to about 500 nucleotides, preferably from about 150 to about 450 nucleotides and more preferably from about 200 to about 400 nucleotides. Thus, it is within the skill of one in the art to design and create a second primer located from about 100 to about 500 nucleotides from the site of a unique sequence. For example, a suitable second primer includes a DNA primer complementary to a sequence in the constant region of a beta chain. Preferred second primers are described herein in the Examples section. Methods to resolve PCR products are well known to those of skill in the art. In addition, methods to quantitate the amount of PCR product produced in a PCR reaction are well known to those of skill in the art. Examples of preferred unique nucleic acid sequences to be identified by PCR include unique sequences contained within SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27 and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80. More preferred unique nucleic acid sequences to be identified by PCR include SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and/or SEQ ID NO:56, or complements thereof. Preferred second primers include primers located in the constant region sequence of a beta chain. More preferred second primers include SEQ ID NO:58 and SEQ ID NO:59, or complements thereof.

In another embodiment, identifying the presence of a T cell receptor having a unique nucleic acid sequence can be achieved by nucleic acid, e.g., DNA, RNA, modified DNA or modified RNA, hybridization techniques using a nucleic acid probe. The hybridization forms a detectable product comprising a hybrid between the nucleic acid and the reagent. A suitable reagent for use with hybridization techniques include nucleic acid probes which are complementary to nucleic acid sequences that include all

or a portion of a unique sequence. The hybridization forms a detectable product comprising a hybrid between the nucleic acid and the reagent. It is within the skill of one in the art to design and produce suitable probes based on sequences of the present invention. The presence of a unique sequence in a sample from an animal is determined by detecting the hybridization of a "unique sequence" probe to that unique sequence in the TCR Vβ nucleic acid molecule. Methods to detect hybridization of a probe are well known to those of skill in the art and include those that allow one to distinguish one Vβ nucleic acid from another. In addition, methods to quantitate the extent of hybridization are well known to those of skill in the art. Preferred unique nucleic acid sequences to be identified by nucleic acid hybridization include unique sequences contained within SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27 and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80. More preferred unique nucleic acid sequences to be identified by nucleic acid hybridization include unique sequences SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and/or SEQ ID NO:56.

A unique amino acid sequence of the present invention can be used to produce antibodies that bind specifically to the portion of a TCR Vß protein that contains the unique amino acid sequence. Such antibodies can be monoclonal or polyclonal, and produced using methods described herein. The antibodies can be used to detect T cells having T cell receptors containing such unique sequences by contacting T cells isolated from an animal with the antibody under appropriate conditions known in the art that enable formation of a complex between an antibody and a T cell receptor in a specific manner, i.e., such that the antibody only binds specifically a particular TCR Vß. The complex between an antibody and a T cell receptor is a detectable product. Methods to

detect such complex formation are known to those of skill in the art and include, for example, using a detectable moiety such as a radioisotope, an enzyme or a fluorescent dye, to detect complex formation. Preferred unique amino acid sequences to be identified using antibodies include antibodies that bind specifically to a unique amino acid sequence contained within SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80. Particularly preferred unique amino acid sequences to be identified using antibodies include antibodies that bind specifically to unique amino acid sequences encoded by a nucleic acid sequence SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56.

In another embodiment, identifying the presence of a T cell receptor having a unique nucleic acid sequence can be achieved by PCR amplification and nucleic acid sequencing techniques. Suitable reagents for use with such techniques include DNA primers which are complementary to common sequences (i.e., sequences shared by two or more TCR Vß molecules) that flank all or a portion of a unique sequence. It is within the skill of one in the art to design and produce suitable primers based on sequences of the present invention. The presence of a unique sequence in a sample from an animal is determined by sequencing the PCR product produced using the common sequence primers and identifying whether one or more nucleic acid sequences are present in the PCR product. An example of such method is described in Example 4 herein. Methods to produce and sequence PCR products are well known to those of skill in the art. T cell expansion is determined by identifying the heterogeneity or homogeneity of nucleic acid sequence displayed in a DNA fingerprint profile of a given sequence in a given sample of PCR products.

Preferred reagents include, but are not limited to, DNA primers or probes complementary to unique nucleic acid sequences contained in SEQ ID NO:1, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27 and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, including DNA primers or probes comprising SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56; or antibodies that bind specifically to an amino acid sequence encoded by a comprising SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and/or SEQ ID NO:56, or to other unique amino acid sequences in larger proteins.

One embodiment of the present invention is a method of detecting T cell expansion in an animal comprising detecting the expansion of a T cell receptor having a unique amino acid sequence within a protein selected from the group consisting of hcVβ3, hcVβ4, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182 proteins by forming detectable products, wherein the increased level of a detectable product compared with another detectable product indicates expansion of the T cells. Preferred Vβ proteins containing unique amino acid sequence include $PCaV\beta3_{127}$, $PCaV\beta3_{111}$, $PCaV\beta3_{110}$, $PCaV\beta4_{128}$, $PCaV\beta4_{113}$, $PCaV\beta4_{109}$, $PCaV\beta12_{134}$, $PCaV\beta12_{111}$, $PCaV\beta12_{115}$, $PCaV\beta72_{133}$, $PCaV\beta72_{113}$, $PCaV\beta72_{114}$, $PCaV\beta21_{130}$, $PCaV\beta21_{108}$, $PCaV\beta21_{116}$, $PCaV\beta54_{135}$, $PCaV\beta54_{114}$, $PCaV\beta54_{116}$, $PCaV\beta182_{128}$, $PCaV\beta182_{110}$ and/or $PCaV\beta182_{109}$.

Examples of samples useful a method of the present invention include samples from an animal that contains T cells. Preferably, a sample to be tested using a method of the present invention comprises blood, synovial fluid, lung lavage, saliva, spleen, thymus, tumors, granulomas, abscesses, edematous fluid, central nervous system fluid.

Preferred animals from which to isolate a sample of the present invention includes a mammal, more preferably a canid and more preferably a dog.

Any method of the present invention can be used to determine the disease state of an animal. Such methods can be used to determine the presence or absence of disease in an animal, including an animal susceptible to disease, an animal suspected of having disease, an animal having disease or an animal being or having been treated for a disease. Examples of specific diseases that can be diagnosed using a method of the present invention include: various forms of cancer, e.g., lymphoma and leukemia; various autoimmune diseases, e.g., rheumatoid arthritis or diabetes; various infectious diseases, such as those caused by viruses, by a yeast, e.g. of the genus Candida, by a parasite, e.g., Trichinella, Leishmania, Toxoplasma, a filariid, a mycobacterium, a protozoan, and by a bacterium; and allergies involving T cells, e.g. allergic dermatitis, atopic dermatitis, allergic bronchitis or allergic rhinitis.

A preferred embodiment of the present invention is a method to diagnose T cell cancer comprising: (a) contacting a sample containing DNA from an animal with a DNA primer that is complementary to a unique nucleic acid sequence within a nucleic acid molecule including nCaV$\beta3_{381}$, nCaV$\beta3_{333}$, nCaV$\beta3_{330}$, nCaV$\beta4_{408}$, nCaV$\beta4_{384}$, nCaV$\beta4_{351}$, nCaV$\beta4_{339}$, nCaV$\beta12_{408}$, nCaV$\beta12_{402}$, nCaV$\beta12_{339}$, nCaV$\beta12_{345}$, nCaV$\beta72_{438}$, nCaV$\beta72_{399}$, nCaV$\beta72_{423}$, nCaV$\beta72_{342}$, nCaV$\beta21_{462}$, nCaV$\beta21_{390}$, nCaV$\beta21_{396}$, nCaV$\beta21_{348}$, nCaV$\beta54_{417}$, nCaV$\beta54_{405}$, nCaV$\beta54_{354}$, nCaV$\beta54_{348}$, nCaV$\beta182_{423}$, nCaV$\beta182_{384}$, nCaV$\beta182_{369}$ or nCaV$\beta182_{327}$; and (b) diagnosing the cancer by determining the amount of DNA containing the unique nucleic acid sequence by comparing the amount so determined with the amount of DNA containing the unique sequence from a normal animal. According to the present invention, "a DNA primer" refers to one or more primers and "a unique nucleic acid sequence" refers to one or more unique nucleic acid sequences.

Another preferred embodiment of the present invention is a method to diagnose T cell cancer comprising: (a) contacting a sample containing DNA from an animal with a DNA primer that is complementary to a unique nucleic acid sequence within nucleic acid molecule selected from the group consisting of nCaV$\beta3_{381}$, nCaV$\beta3_{333}$, nCaV$\beta3_{330}$, nCaV$\beta4_{408}$, nCaV$\beta4_{384}$, nCaV$\beta4_{351}$, nCaV$\beta4_{339}$, nCaV$\beta12_{408}$, nCaV$\beta12_{402}$, nCaV$\beta12_{339}$,

$nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$,$nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ or $nCaV\beta182_{327}$; and (b) diagnosing cancer by quantitatively determining the ratio of DNA containing each of the unique nucleic acid sequences and by comparing the amount of DNA containing each of the unique sequence with each other.

The present invention also includes a kit comprising one or more reagents of the present invention. Preferred reagents include those which can differentiate TCR V beta proteins or nucleic acid molecules that encode such proteins. Preferred TCR V beta proteins include hcVβ2, hcVβ3, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182, with $PCaV\beta3_{127}$, $PCaV\beta3_{111}$, $PCaV\beta3_{110}$, $PCaV\beta4_{128}$, $PCaV\beta4_{113}$, $PCaV\beta4_{109}$, $PCaV\beta12_{134}$, $PCaV\beta12_{111}$, $PCaV\beta12_{115}$, $PCaV\beta72_{133}$, $PCaV\beta72_{113}$, $PCaV\beta72_{114}$, $PCaV\beta21_{130}$, $PCaV\beta21_{108}$, $PCaV\beta21_{116}$, $PCaV\beta54_{135}$, $PCaV\beta54_{114}$, $PCaV\beta54_{116}$, $PCaV\beta182_{128}$, $PCaV\beta182_{110}$ and/or $PCaV\beta182_{109}$ being more preferred and proteins comprising SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80 and/or proteins encoded by the complement of a nucleic acid sequence including SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56 being even more preferred. Preferred TCR V beta nucleic acid molecules include hcVβ2, hcVβ3, hcVβ12, hcVβ72, hcVβ21, dtb54 or dtb182, with $nCaV\beta3_{381}$, $nCaV\beta3_{333}$, $nCaV\beta3_{330}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta4_{351}$, $nCaV\beta4_{339}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta12_{339}$, $nCaV\beta12_{345}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta72_{423}$, $nCaV\beta72_{342}$, $nCaV\beta21_{462}$, $nCaV\beta21_{390}$, $nCaV\beta21_{396}$, $nCaV\beta21_{348}$, $nCaV\beta54_{417}$, $nCaV\beta54_{405}$,$nCaV\beta54_{354}$, $nCaV\beta54_{348}$, $nCaV\beta182_{423}$, $nCaV\beta182_{384}$, $nCaV\beta182_{369}$ and/or $nCaV\beta182_{327}$ being more preferred and nucleic acid molecules comprising sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ

ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27,SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, a complement of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and/or a nucleic acid molecule that encodes a protein having an amino acid sequence including SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80, and complements thereof, being even more preferred. A kit of the present invention can include mixtures of reagents disclosed herein.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.* and Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, NY, and related references. It should also be noted that since nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, that the nucleic acid and deduced protein sequences presented herein represent apparent nucleic acid sequences of the nucleic acid molecules encoding TCR Vβ proteins of the present invention.

Example 1

This example describes the isolation and sequencing of canine T cell receptor (TCR) Vβ nucleic acid molecules.

Canine TCR Vβ nucleic acid molecules were produced as follows. RNA was purified from mitogen activated canine peripheral blood lymphocytes and resuspended in water at about 2.5 X 10^5 cell equivalent/microliter (μl). About 5 μl of RNA and random hexanucleotide primers were used to synthesize cDNA using the First Strand cDNA Synthesis™ kit (available from Pharmacia, Uppsala, Sweden). TCR Vβ genes were selectively PCR amplified from the cDNA using degenerate oligonucleotides designed using the conserved sequence motifs WYRQ and Y(Y/F)CA of T and B cell antigen receptors (Rast and Litman, *Proc. Natl. Acad. Sci. USA*, vol. 91, p. 9248, 1994). The degenerate primer FR2, having the nucleic acid sequence 5' CCG AAT TCT GGT A(TC) C(GA) NCA 3' (SEQ ID NO:81) was used in combination with either the FR3A primer, having the nucleic acid sequence 5' CGG ATC CGC (GA)CA (GA)TA (GA)T A 3' (SEQ ID NO:82) or the primer FR3B, having the nucleic acid sequence 5' CGG ATC CGC (GA)CA (GA)A A(GA)T A 3' (SEQ ID NO:83). First round PCR reactions were performed in about 50μl of 50 mM KCl, 10 mM Tris-HCl, 0.01% gelatin (pH 8.3), 3.5 mM $MgCl_2$, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dNTP), about 1μM of each primer and about 2.0 units Taq polymerase (available from Perkin-Elmer). This reaction mixture was created after attempts using published methods failed. Moreover, 20 additional cycles of PCR using about 5μl of the first round PCR sample as template under identical conditions were needed to obtain useful second round PCR product. No PCR products were identified after 30 cycles. Second round PCR products were resolved by loading about 15 μl of the second round PCR product onto a 1.0% agarose gel in TBE buffer and staining the gel with ethidium bromide.

No PCR product was obtained using the FR2 primer combined with the FR3A primer. Several bands of DNA were obtained using the FR2 primer combined with the FR3B primer, one of which migrated at a predicted size of about 190 base pair (bp). This band was excised, and the DNA was purified using Qiaquick gel extraction kit (available from Qiagen, Chatsworth, CA) according to the manufacturer's instructions. The purified DNA was cloned into the pCR 2.1 vector (available from Invitrogen, San Diego, CA), and used to transform DH5α *E. coli* cells (available from Invitrogen). The transformed colonies were grown overnight in about 2 ml LB media containing about 100 μg/ml ampicillin. Plasmid DNA was purified using BioRad's Quantum Prep™

mini-prep kit (available from BioRad, Hercules, CA). Inserted sequence in 11 different plasmid samples were subjected to DNA sequence analysis using standard sequencing methods. Three different nucleic acid sequences were obtained. The 3 different nucleic acid molecules are referred to herein as hcvb3, hcvb4 and hcvb12.

The sequences obtained above indicated that incomplete nucleic acid molecules were obtained using the foregoing PCR procedure. To obtain more complete clones of the PCR products, primers were designed using the nucleic acid sequences obtained above, including primer Phcvb3, having the nucleic acid sequence 5' CCA GAC CTG GGT CTT GTC G 3' (SEQ ID NO:84), primer Phcvb4, having the nucleic acid sequence 5' CTC TGT CCT GGG AGC TGA C 3' (SEQ ID NO:85), and primer Phcvb12, having the nucleic acid sequence 5' TTG TTT GAT CTA GAG ACT GTG 3' (SEQ ID NO:86). Each primer was then used in combination with the 5' vector primer T3 (available from available from Stratagene Cloning Systems, La Jolla, CA) to amplify PCR products representing Vβ genes from a canine PBL cDNA library generated in the λ Zap II vector (available from Stratagene Cloning Systems, La Jolla, CA). The *C. familiaris* mitogen activated PBMC cDNA library was constructed in the Uni-ZAP® XR vector (available from Stratagene Cloning Systems), using Stratagene's ZAP-cDNA® Synthesis Kit and the manufacturer's protocol. The mRNA was isolated from *C. familiaris* peripheral blood mononuclear cells 4 hours after they were activated by a polyclonal activating agent in culture. PCR reaction were performed using the following conditions: Taq activation was performed at about 95°C for about 10 min, about 94°C for about 30 sec., about 58°C for about 30 sec., and about 72°C for about 1 min. for about 35 cycles, then clonal extension was performed at about 72°C for about 5 min.; PCR was performed in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, pH 3, 25 mM $MgCl_2$, about 1 unit Amplitaq Gold™ (available from Perkin-Elmer Cetus), about 200 μM dNTP's and about 1 μM primer.

The resulting PCR products were gel purified, cloned and sequenced using standard methods. The resulting nucleic acid sequences were aligned with the nucleic acid sequences obtained from the first set of PCR products to obtain a more complete sequence of hcvb3, hcvb4 and hcvb12. Since the PCR primers used to generate the first set of PCR products were degenerate primers, the nucleic acid sequences of hcvb3,

hcvb4 and hcvb12 at the degenerate primer sites were ambiguous. To determine the sequence in the area of the degenerate primers, PCR primers corresponding to the newly derived extreme 5' ends of the hcvb3, hcvb4 and hcvb12 nucleic acid molecules were designed. Primer 5'Phcvb3, having the nucleic acid sequence 5' ATC GGA CTC CTC TGT GGT GT 3' (SEQ ID NO:87), primer 5'hcvb4, having the nucleic acid sequence 5' ACG GTG AAG GGC TAG CAC CT 3' (SEQ ID NO:88) and primer 5'hcvb12, having the nucleic acid sequence 5' GCT GAA ATG GCC ACC GGC GT 3' (SEQ ID NO:89), each were used in combination with a primer specific for a sequence in the constant region of a TCR beta chain (SED ID NO:57) in PCR reactions using the cDNA library described above. The resulting PCR products were purified, cloned into PCR2.1 vector (available from Invitrogen)and sequenced using standard methods.

A. A first clone (hcVβ3) was isolated, referred to herein as $nCaV\beta3_{381}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:1. SEQ ID NO:1 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. Translation of SEQ ID NO:1 suggests that nucleic acid molecule $nCaV\beta3_{381}$ encodes a TCR Vβ protein of about 127 amino acids, denoted herein as $PCaV\beta3_{127}$, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having a first codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:1 and a last codon spanning from nucleotide 379 through nucleotide 381 of SEQ ID NO:1. The putative signal sequence extends from nucleotide 1 to nucleotide 51 of SEQ ID NO:1. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as $PCaV\beta3_{110}$, contains about 110 amino acids, extending from residue 18 through residue 127 of SEQ ID NO:2. The nucleic acid molecule encoding $PCaV\beta3_{110}$ is denoted herein as $nCaV\beta3_{330}$, extending from nucleotide 52 through nucleotide 381 of SEQ ID NO:1.

Comparison of nucleic acid sequence SEQ ID NO:1 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 69% identity, between SEQ ID NO:1 and a human TCR β chain gene (Genbank Accession No. Z223040). Comparison of amino acid sequence SEQ ID NO:2 with amino acid sequences reported in GenBank indicates that SEQ ID NO:2 showed the

most homology, i.e., about 65% identity, between SEQ ID NO:2 and an *Ovis aries* TCR Vβ chain protein (Genbank Accession No. gi 2665554).

B. A second clone (hcVβ4) was isolated, referred to herein as nCaV$\beta 4_{408}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:4. SEQ ID NO:4 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:4 is represented herein by SEQ ID NO:6. Translation of SEQ ID NO:4 suggests that nucleic acid molecule nCaV$\beta 4_{408}$ encodes a TCR Vβ protein of about 128 amino acids, denoted herein as PCaV$\beta 4_{128}$, the amino acid sequence of which is presented in SEQ ID NO:5, assuming an open reading frame having an initiation codon spanning from nucleotide 24 through nucleotide 26 of SEQ ID NO:4 and a last codon spanning from nucleotide 405 through nucleotide 407 of SEQ ID NO:4. The coding region encoding PCaV$\beta 4_{128}$ is presented herein as nCaV$\beta 4_{384}$, which has the nucleotide sequence SEQ ID NO:6 (the coding strand) and SEQ ID NO:7 (the complementary strand). The putative signal sequence extends from nucleotide 25 to nucleotide 69 of SEQ ID NO:4. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaV$\beta 4_{113}$, contains about 113 amino acids, extending from residue 60 through residue 128 of SEQ ID NO:5. The nucleic acid molecule encoding PCaV$\beta 4_{113}$ is denoted herein as nCaV$\beta 4_{339}$, extending from nucleotide 70 through nucleotide 408 of SEQ ID NO:4.

Comparison of nucleic acid sequence SEQ ID NO:4 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:4 showed the most homology, i.e., about 75% identity, between SEQ ID NO:4 and a human TCR β chain gene (Genbank Accession No. M97713). Comparison of amino acid sequence SEQ ID NO:5 with amino acid sequences reported in GenBank indicates that SEQ ID NO:5 showed the most homology, i.e., about 69% identity, between SEQ ID NO:5 and an *Ovis aries* TCR Vβ chain protein (Genbank Accession No. gi 2665558).

C. A third clone (hcVβ12) was isolated, referred to herein as nCaV$\beta 12_{408}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:9. SEQ ID NO:9 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:9 is represented herein by SEQ ID NO:11. Translation of SEQ ID NO:9 suggests that nucleic acid molecule nCaV$\beta 12_{408}$ encodes a

TCR Vβ protein of about 134 amino acids, denoted herein as PCaVβ12_{134}, the amino acid sequence of which is presented in SEQ ID NO:10, assuming an open reading frame having an initiation codon spanning from nucleotide 7 through nucleotide 9 of SEQ ID NO:9 and a last codon spanning from nucleotide 406 through nucleotide 408 of SEQ ID NO:9. The coding region encoding PCaVβ12_{134} is presented herein as nCaVβ12_{402}, which has the nucleotide sequence SEQ ID NO:12 (the coding strand) and SEQ ID NO:13 (the complementary strand). The putative signal sequence extends from nucleotide 7 to nucleotide 63 of SEQ ID NO:9. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ12_{115}, contains about 115 amino acids, extending from residue 20 through residue 134 of SEQ ID NO:10. The nucleic acid molecule encoding PCaVβ12_{115} is denoted herein as nCaVβ12_{345}, extending from nucleotide 64 through nucleotide 408 of SEQ ID NO:9.

Comparison of nucleic acid sequence SEQ ID NO:9 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:9 showed the most homology, i.e., about 72% identity, between SEQ ID NO:9 and a *Macaca mulatta* TCR β chain mRNA (Genbank Accession No. U04578). Comparison of amino acid sequence SEQ ID NO:10 with amino acid sequences reported in GenBank indicates that SEQ ID NO:10 showed the most homology, i.e., about 57% identity, between SEQ ID NO:10 and a human TCR Vβ protein (Genbank Accession No. I38312).

Example 2

This example describes the isolation and sequencing of two additional canine TCR Vβ nucleic acid molecules.

Two canine TCR Vβ nucleic acid molecules were PCR amplified from the canine PBL cDNA library described above in Example 1. A pair of primers was used to amplify DNA from the cDNA library. The 5' vector primer T3, described in Example 1, was used in combination with primer Phcvb21, having the nucleic acid sequence 5' CTG TTG CCC ACG TTA GAG G 3' (SEQ ID NO:90) or primer Phcvb72, having the nucleic acid sequence 5' TTA CTG AAC TGC TGC ACT G 3' (SEQ ID NO:91). PCR reaction were performed using the following conditions: Taq activation was performed at about 95°C for about 10 min., about 94°C for about 30 sec., about 60°C for about 30

sec., about 72°C for about 1 min. for about 35 cycles; then clonal extension was performed at about 72°C for about 5 min.; PCR was performed in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, pH 3, 3 mM $MgCl_2$, about 1 unit Amplitaq Gold, about 200 μM dNTP's and about 1 μM primer. The resultant PCR products obtained using standard PCR conditions (e.g., Sambrook et al., *ibid.*), were gel purified, cloned and sequenced. The PCR products are referred to herein as hcvb21 or hcvb72, respectively.

The sequences obtained were compared with sequences disclosed in Ito et al., *Immunogenetics*, vol. 38, p. 60, 1993 or Takano et al., *Immunogenetics*, vol. 40, p. 246, 1994. The PCR products hcvb21 or hcvb72 were found to contain more 5' nucleic acid sequence than that disclosed in the above-referenced publications. To obtain more complete nucleic acid molecules containing the V, D and J regions, PCR amplification was performed using primers designed from the 5' sequence obtained from hcvb21 or hcvb72 nucleic acid molecules. Primer 5'Phcvb21, having the nucleic acid sequence 5' GCT GCA GGA TTC GGC ACG AG 3' (SEQ ID NO:92) or primer 5'Phcvb72, having the nucleic acid sequence 5' TAC GAC TGT CAG CTT GGT CC 3' (SEQ ID NO:93), each were used in conjunction with a TCR beta constant region primer (SEQ ID NO:57) to amplify these sequences from mRNA prepared from canine concavalin A (ConA) activated PBMC. The PCR products were cloned and sequenced using standard methods.

A. The clone hcVβ21 was isolated, referred to herein as $nCaV\beta21_{462}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:19. SEQ ID NO:19 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:19 is represented herein by SEQ ID NO:21. Translation of SEQ ID NO:19 suggests that nucleic acid molecule $nCaV\beta21_{462}$ encodes a TCR Vβ protein of about 130 amino acids, denoted herein as $PCaV\beta21_{130}$, the amino acid sequence of which is presented in SEQ ID NO:20, assuming an open reading frame having an initiation codon spanning from nucleotide 73 through nucleotide 75 of SEQ ID NO:19 and a last codon spanning from nucleotide 460 through nucleotide 462 of SEQ ID NO:19. The coding region encoding $PCaV\beta21_{130}$ is presented herein as $nCaV\beta21_{390}$, which extends from nucleotide 73 to nucleotide 462 of SEQ ID NO:19.

The putative signal sequence extends from nucleotide 73 to nucleotide 114 of SEQ ID NO:19. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ21$_{116}$, contains about 116 amino acids, extending from residue 15 through residue 130 of SEQ ID NO:19. The nucleic acid molecule encoding PCaVβ21$_{116}$ is denoted herein as nCaVβ21$_{348}$, extending from nucleotide 115 through nucleotide 462 of SEQ ID NO:19.

A comparison of SEQ ID NO:19 with the DNA sequence DTCRB21 described in Ito et al., *ibid.* indicated that SEQ ID NO:19 is 221 nucleotides longer than at the 5'end of DTCRB21. The sequences of SEQ ID NO:19 and DTCR21 overlap by 176 nucleotides with 100% identity, corresponding to nucleotides 222 through 398 of SEQ ID NO:19. An alignment of SEQ ID NO:19 with DTCR21 is shown in Table 1.

TABLE 1. Nucleotide Sequence Comparison of hcvb21 and DTCRB21

DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~
SEQ ID NO:19	GCTGCAGGAT	TCGGCACGAG	GCGTGGTCAT	ATCTATCTTG	AGAGAGGTAT
DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~
SEQ ID NO:19	GGTATGAGGC	CATCACCTGA	AGATGCTGAT	GCTTCTGCTG	CTCCTGGGGC
DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~
SEQ ID NO:19	CCAGCTCTGG	ACTCGGTGCC	CTCGTCTTCC	AGGCGCCCAG	CACAATGATC
DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~
SEQ ID NO:19	TGTAAGAGCG	GAGCCACCGT	GCAGATCCAG	TGTCAAACAG	TGGACCTTCA
DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~TCGCCAGCT	CCCGAAGCAG	GGCCTTACCC
SEQ ID NO:19	AGCCACAACC	GTGTTTTGGT	ATCGCCAGCT	CCCGAAGCAG	GGCCTTACCC
DTCRB21	TTATGGTGAC	CTCTAACGTG	GGCAACAGTG	CTACACACGA	GCAGGGGTTC
SEQ ID NO:19	TTATGGTGAC	CTCTAACGTG	GGCAACAGTG	CTACACACGA	GCAGGGGTTC
DTCRB21	CCTGCAGCCA	AGTTCCCTGT	TAACCACCCA	AACCTCACGT	TTTCCTCCCT
SEQ ID NO:19	CCTGCAGCCA	AGTTCCCTGT	TAACCACCCA	AACCTCACGT	TTTCCTCCCT
DTCRB21	GATGGTGACG	AGTTCAGGTC	CTGGAGACAG	CGGCCTCTAC	TTCTGTGGCT
SEQ ID NO:19	GATGGTGACG	AGTTCAGGTC	CTGGAGACAG	CGGCCTCTAC	TTCTGTGGTG
DTCRB21	ACC...TACA	GGGCGCGCGC	TACGAGCAGT	ATTTCGGCGC	CGGCACCAGG
SEQ ID NO:19	TTCGGGCGTA	TGGTGGGAAC	TCGCCCCTCT	ACTTTGGAAC	AGGCACCAGG
DTCRB21	CTCACGGTCC	TC			
SEQ ID NO:19	CTCACCGTGA	CA			

The amino acid sequence SEQ ID NO:20 is 50 amino acids longer at the 5'end than the amino acid sequence encoded by DTCR21. An alignment of SEQ ID NO:20 with the amino acid sequence encoded by DTCR21 is shown in Table 2.

TABLE 2. Amino Acid Sequence Comparison of hcvb21 and DTCR21.

SEQ ID NO:20	MLMLLLLLGP	SSGLGALVFQ	APSTMICKSG	ATVQIQCQTV	DLQATTVFWY
DTCRB21	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~	~~~~~~~~~~
SEQ ID NO:20	RQLPKQGLTL	MVTSNVGNSA	THEQGFPAAK	FPVNHPNLTF	SSLMVTSSGP
DTCRB21	RQLPKQGLTL	MVTSNVGNSA	THEQGFPAAK	FPVNHPNLTF	SSLMVTSSGP
SEQ ID NO:20	GDSGLYFCGV	RAYGGNSPLY	FGTGTRLTVT		
DTCRB21	GDSGLYFCGY	.LQGARYEQY	FGAGTRLTVL		

The amino acid sequence SEQ ID NO:20 is 50 amino acids longer at the 5'end than the amino acid sequence encoded by DTCR21. An alignment of SEQ ID NO:20 with the amino acid sequence encoded by DTCR21 is shown in Table 2.

Comparison of nucleic acid sequence SEQ ID NO:19 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:19 showed the most homology, i.e., about 38% identity, between SEQ ID NO:19 and a dog TCR β chain gene (Genbank Accession No. M97510). Comparison of amino acid sequence SEQ ID NO:20 with amino acid sequences reported in GenBank indicates that SEQ ID NO:20 showed the most homology, i.e., about 60% identity, between SEQ ID NO:20 and DTCR21 protein.

B. The clone hcVβ72 was isolated, referred to herein as $nCaV\beta72_{438}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:98. SEQ ID NO:98 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:98 is represented herein by SEQ ID NO:100. Translation of SEQ ID NO:98 suggests that nucleic acid molecule $nCaV\beta72_{438}$ encodes a TCR Vβ protein of about 133 amino acids, denoted herein as $PCaV\beta72_{133}$, the amino acid sequence of which is presented in SEQ ID NO:15, assuming an open reading frame having an initiation codon spanning from nucleotide 40 through nucleotide 42 of SEQ ID NO:98 and a last codon spanning from nucleotide 436 through nucleotide 438 of SEQ ID NO:98. The coding region encoding $PCaV\beta72_{133}$ is presented herein as $nCaV\beta72_{399}$, which has the nucleotide sequence SEQ ID NO:17 (the coding strand) and

SEQ ID NO:18 (the complementary strand). The putative signal sequence extends from nucleotide 40 to nucleotide 96 of SEQ ID NO:98. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ72_{114}, contains about 114 amino acids, extending from residue 20 through residue 133 of SEQ ID NO:15. The nucleic acid molecule encoding PCaVβ72_{114} is denoted herein as nCaVβ72_{342}, extending from nucleotide 142 through nucleotide 438 of SEQ ID NO:19.

A comparison of SEQ ID NO:98 with the DNA sequence DTB72 described in Takano et al., *ibid.* indicated that SEQ ID NO:98 differs substantially from the published canine TCR Vβ sequence. A comparison between SEQ ID NO:98 and DTB72 is shown in Table 3.

TABLE 3. Nucleotide Sequence Comparison of hcvb72 and DTB72

DTB72					~~~~~
SEQ ID NO:98					CACGA
DTB72	~~~~~~~~~~	~~~~~~CAGC	TTCCCAGGGC	TGCCATGGGC	TCCAGGCTTC
SEQ ID NO:98	GGAGCGGGGA	GGCTATCAGC	TTCCCAGGGC	TGCCATGGGC	TCCAGGCTTC
DTB72	TCTGCTGTGT	GGCCCTTTTC	TCCTGGGAGC	CGGCCCCCGT	GGAGTCTGAG
SEQ ID NO:98	TCTGCTGTGT	GGCCCTTTGT	CTCCTGGGAG	CCGGCCCCGT	GGAGTCTGAG
DTB72	GTCATCCAAA	CTCCAAGACA	CATGATCAAA	GTCAAGAGGA	CAGACAGTGA
SEQ ID NO:98	GTCATCCAAA	CTCCAAGACA	CATGATCAAA	G.CAAGAGGA	CAGACAGTGA
DTB72	CC...TGAGA	TGTCCTTATC	TCTGGACA.C	TATCTGTGTA	CTGGTACCAA
SEQ ID NO:98	CCCTGAGATG	TTCCCTTATC	TCTGGACACC	TATCTGTGTA	CTGGTACCAA
DTB72	CAGGCCTTGA	TGGTCCGTTT	ACCGGTTTCT	CATTCAGT..	C
SEQ ID NO:98	CAGGCCCTG.	GGCCAGGGTC	CCCGGTTTCT	CATTCAGTAT	TACAATAGGG
DTB72	ATCATAGTCA	AAAAAGAAAC	ATCCGGTCAA	GATTCTCAGT	GCAGCAGTTC
SEQ ID NO:98	AAGAGAGAGA	CAAAGGAGAC	ATCCCGGCAA	GATTCTCAGT	GCAGCAGTTC
DTB72	AGTAACTACA	GCATCCCAGC	TTGAGATGAA	CTCCCTGGAG	CCAGGAGACT
SEQ ID NO:98	AGTAACTACA	GC.TCCCAGC	TGGAGATGAA	CTCCCTGGAG	CCAGGAGACT
DTB72	CAGCCCTATA	TCTCTGTGCC	AGCAGC...G	GGTACAGTGA	GAGCTACGAG
SEQ ID NO:98	CAGCCCTATA	TCTCTGTGCC	AGCAGCTTAG	ATGCGTTCGA	CGCGGGGCAG
DTB72	CGGTATTTCG	GAGCCGGCAC	CAGGCTCACG	GTCCTC	
SEQ ID NO:98	CTGTACTTCG	GGGCCGGTTC	CAAGCTGGCC	GTGCTG	

Comparison of SEQ ID NO:98 with DTB72 indicates that the identity between the two sequences is about 90%, when determined using the Compare function by maximum matching within the program DNAsis™ Version 2.1. A comparison of the amino acid

sequence SEQ ID NO:15 and the amino acid sequence encoded by DTB72 indicates that the identity between the two sequences is only 57%, when determined using the Compare function by maximum matching within the program DNAsis™ Version 2.1. An alignment of the two sequences is shown in Table 4.

TABLE 4. Amino Acid Sequence Comparison of hcvb72 and DTB72

```
SEQ ID NO:15   MGSRLLCCVA LCLLGAGPVE SEVIQTPRHM IKARGQTVTL RCSLISGHLS
DTB72          MGSRLLCCVA LFSWEPAPVE SEVIQTPRHM IKVKRTDSDL RCPYL.WTLS

SEQ ID NO:15   VYWYQQAL.G QGPRFLIQYY NREERDKGDI PARFSVQQFS NYSSQLEMNS
DTB72          VYWYQQALMV RLPVSHSVII VKKETSGQDS QCSSSV.... TTASQLEMNS

SEQ ID NO:15   LEPGDSALYL CASSLDAFDA GQLYFGAGSK LAVL
DTB72          LEPGDSALYL CASS.GYSES YERYFGAGTR LTVL
```

The comparison of the two sequences indicates that SEQ ID NO:98 and DTB72 encode different proteins.

Comparison of nucleic acid sequence SEQ ID NO:19 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:19 showed the most homology, i.e., about 60% identity, between SEQ ID NO:19 and a feline leukemia virus transduced TCR β chain gene (Genbank Accession No. X05155). Comparison of amino acid sequence SEQ ID NO:20 with amino acid sequences reported in GenBank indicates that SEQ ID NO:20 showed the most homology, i.e., about 65% identity, between SEQ ID NO:20 and a a feline leukemia virus transduced TCR β chain protein (Genbank Accession No. RWMVTV).

Example 3

This example describes the production of two canine TCR Vβ nucleic acid molecules.

Two canine TCR Vβ nucleic acid molecules were PCR amplified from the canine PBL cDNA library described above in Example 1. A pair of primers was used to amplify DNA from the cDNA library. The 5' vector primer T3, described in Example 1, was used in combination with primer Pdtb54, having the nucleic acid sequence 5' CTT TTG CTG GGA TCT GCT GA 3' (SEQ ID NO:94) or primer Pdtb182, having the nucleic acid sequence 5' CAG TTG CTT AG GTC TTG CT 3' (SEQ ID NO:95). The resultant PCR products obtained using standard PCR conditions (e.g., Sambrook et al.,

ibid.), were gel purified, cloned and sequenced. The PCR products are referred to herein as dtb54 or dtb182, respectively.

The sequences obtained were compared with sequences disclosed in Takano et al., *ibid.* The PCR products dtb54 or dtb182 were found to contain more 5' nucleic acid sequence than that disclosed in the above-referenced publication. To obtain more complete nucleic acid molecules containing the V, D and J regions, PCR amplification was performed using primers designed from the 5' sequence obtained from dtb54 or dtb182 nucleic acid molecules. Primer 5'Pdtb54, having the nucleic acid sequence 5' CAC GAG CCT GCC ATG TGC CC 3' (SEQ ID NO:96) or primer 5'Pdtb182, having the nucleic acid sequence 5' GGC ACG AGC ACT GAG GAC CA 3' (SEQ ID NO:97), each were used in conjunction with a TCR beta constant region primer (SEQ ID NO:57) to amplify these sequences from mRNA prepared from canine concavalin A (ConA) activated PBMC. The PCR products were cloned and sequenced using standard methods.

A. The clone dtb54 was isolated, referred to herein as nCaVβ54$_{417}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:22. SEQ ID NO:22 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:22 is represented herein by SEQ ID NO:24. Translation of SEQ ID NO:22 suggests that nucleic acid molecule nCaVβ54$_{417}$ encodes a TCR Vβ protein of about 135 amino acids, denoted herein as PCaVβ54$_{135}$, the amino acid sequence of which is presented in SEQ ID NO:23, assuming an open reading frame having an initiation codon spanning from nucleotide 13 through nucleotide 15 of SEQ ID NO:22 and a last codon spanning from nucleotide 415 through nucleotide 417 of SEQ ID NO:22. The coding region encoding PCaVβ54$_{135}$ is presented herein as nCaVβ54$_{405}$, which extends from nucleotide 13 to nucleotide 417 of SEQ ID NO:22. The putative signal sequence extends from nucleotide 13 to nucleotide 69 of SEQ ID NO:22. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ54$_{116}$, contains about 116 amino acids, extending from residue 20 through residue 135 of SEQ ID NO:22. The nucleic acid molecule encoding PCaVβ54$_{116}$ is denoted herein as nCaVβ54$_{348}$, extending from nucleotide 70 through nucleotide 417 of SEQ ID NO:22.

A comparison of SEQ ID NO:22 with the DNA sequence DTB54 described in Takano et al., *ibid.* indicated that the sequences are substantially similar except for 12 additional nucleotides at the 5'end and an additional amino acid at residue 55 in SEQ ID NO:22.

B. The clone dtb182 was isolated, referred to herein as nCaVβ182$_{423}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:25. SEQ ID NO:25 includes the V, D and J regions of the sequenced PCR product. The complement of SEQ ID NO:25 is represented herein by SEQ ID NO:27. Translation of SEQ ID NO:25 suggests that nucleic acid molecule nCaVβ182$_{423}$ encodes a TCR Vβ protein of about 128 amino acids, denoted herein as PCaVβ182$_{128}$, the amino acid sequence of which is presented in SEQ ID NO:26, assuming an open reading frame having an initiation codon spanning from nucleotide 40 through nucleotide 43 of SEQ ID NO:25 and a last codon spanning from nucleotide 421 through nucleotide 423 of SEQ ID NO:25. The coding region encoding PCaVβ182$_{128}$ is presented herein as nCaVβ182$_{384}$, which extends from nucleotide 40 to nucleotide 423 of SEQ ID NO:25. The putative signal sequence extends from nucleotide 40 to nucleotide 96 of SEQ ID NO:25. The proposed mature protein (i.e., canine TCR Vβ protein from which the signal sequence has been cleaved), denoted herein as PCaVβ182$_{109}$, contains about 109 amino acids, extending from residue 20 through residue 128 of SEQ ID NO:25. The nucleic acid molecule encoding PCaVβ182$_{109}$ is denoted herein as nCaVβ182$_{327}$, extending from nucleotide 97 through nucleotide 423 of SEQ ID NO:25.

A comparison of SEQ ID NO:25 with the DNA sequence DTB182 described in Takano et al., *ibid.* indicated that the sequences are substantially similar except for 84 additional nucleotides at the 5'end of SEQ ID NO:25.

<u>Example 4</u>

This example describes the identification of unique TCR Vβ sequences by designing PCR primers that distinguish between different (TCR) Vβ nucleic acid molecules.

Seven different primers for PCR reactions were designed to amplify DNA from seven different TCR Vβ nucleic acid molecules. The primers were designed based on

the nucleic acid sequences SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:98, SEQ ID NO:19, SEQ ID NO:22 or SEQ ID NO:25. The inventors discovered unique sequences for each of the foregoing nucleic acid molecules that are not shared between each of the different molecules. In addition, the inventors discovered which primers designed based on those unique sequences, and under what specific conditions, did not cross-prime between the different nucleic acid molecules. For some Vβ genes, several different Vβ primers to unique sequences had to be tested in order to find one that was specific to only one Vβ gene. Some of the primers that were designed include: primer hcVβ3 unique, having the nucleic acid sequence 5' CGA CAA GAC CCA GGT CTG G 3' (SEQ ID NO:50), complementary to nucleotides 157 to 175 of SEQ ID NO:1; primer hcVβ4 unique, having the nucleic acid sequence 5' GTC AGC TCC CAG GAC AGA G 3' (SEQ ID NO:51), complementary to nucleotides 176 to 194 of SEQ ID NO:4; primer hcVβ12 unique, having the nucleic acid sequence 5' CAT GAC CTG GGA CAT GGG C 3' (SEQ ID NO:52), complementary to nucleotides 172 to 190 of SEQ ID NO:9; primer hcVβ72 unique, having the nucleic acid sequence 5' GAG ATG TTC CCT TAT CT CTGG 3' (SEQ ID NO:53), complementary to nucleotides 201 to 226 of SEQ ID NO:98; primer hcVβ21 unique, having the nucleic acid sequence 5' CCT CTA ACG TGG GCA ACA G 3' (SEQ ID NO:54), complementary to nucleotides 260 to 278 of SEQ ID NO:19; primer dtb54 unique, having the nucleic acid sequence 5' TCA GCA GAT CCC AGC AAA AG 3' (SEQ ID NO:55), complementary to nucleotides 174 to 193 of SEQ ID NO:22; and primer dtb182 unique, having the nucleic acid sequence 5' AGC AAG ACC TCA AGC AAC TG 3' (SEQ ID NO:56), complementary to nucleotides 203 to 222 of SEQ ID NO:25.

The ability of each primer to specifically prime a specific TCR Vβ gene was tested by performing PCR reactions using each of the above primers in combination with a Vβ constant region primer including: primer Cβ1, having the nucleic acid sequence 5' GTG ACC TTC TGC AGA TCC TC 3' (SEQ ID NO:57); primer Cβ2, having the nucleic acid sequence 5' AGC TCA GCT CCA CGT GGT C 3' (SEQ ID

NO:58); or primer Cβ3, having the nucleic acid sequence 5' TGC TGA ACC CAC TCG TGA C 3' (SEQ ID NO:59).

The specificity of these primers was first tested using 7 different DNA plasmids that contained nucleic acid molecules comprising either SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:98, SEQ ID NO:19, SEQ ID NO:22 or SEQ ID NO:25 as templates for different PCR reaction. Each unique Vβ primer was used to amplify DNA from each template. PCR reactions were performed using the following conditions: Taq activation was performed at about 95°C for about 10 min., about 94°C for about 30 sec., about 60°C for about 30 sec., about 72°C for about 1 min. for about 35 cycles; then clonal extension was performed at about 72°C for about 5 min.; PCR was performed in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, pH 3, 3 mM $MgCl_2$, about 1 unit Amplitaq Gold, about 0.1 μM dNTP's and about 0.4 μM primer. The results indicated that PCR products were only present when the correct template and primer combination was used. As such, only PCR products were present when primer hcVβ3 unique was used with a plasmid containing SEQ ID NO:1, primer hcVβ4 unique was used with a plasmid containing SEQ ID NO:4, primer hcVβ12 unique was used with a plasmid containing SEQ ID NO:9, primer hcVβ72 unique was used with a plasmid containing SEQ ID NO:98, primer hcVβ21 unique was used with a plasmid containing SEQ ID NO:19, primer dtb54 unique was used with a plasmid containing SEQ ID NO:22 or primer dtb182 unique was used with a plasmid containing SEQ ID NO:25.

The ability of the Vβ unique primers to prime DNA of the predicted size for different Vβ genes using cDNA from a canine ConA activated T cell population as template material was tested. The same primers and PCR amplification conditions described immediately above were used in these experiments. The resulting PCR products were resolved by electrophoresis on an about 1.2% LE agarose gel in TBE buffer and stained with ethidium bromide. The results shown in Fig. 1A indicated that all seven Vβ unique primers were able to prime DNA fragments of the correct size. To confirm that only a specific Vβ gene was amplified by each Vβ primer, the DNA bands shown in Fig. 1A were extracted from the gel and cloned. Different clones containing

DNA from the different bands were sequenced. The sequencing results indicated that each Vβ unique primer correctly primed only one Vβ gene containing sequence complementary to that primer.

Example 5

This example describes a method for identifying T cell expansion.

About 10^5 lymph node cells or PBMC were isolated from 2 dogs known to have lymphoma (Haynes and Stoll) and a control dog. cDNA samples from the cells were used as templates in separate PCR reactions using primer hcVβ3 unique, primer hcVβ4 unique, primer hcVβ12 unique, primer hcVβ72 unique, primer hcVβ21 unique, primer dtb54 unique or primer dtb182 unique, in combination with Cβ3 primer (SEQ ID NO:59). PCR reactions were performed using the following conditions: Taq activation was performed at about 95°C for about 10 min., about 94°C for about 30 sec., about 60°C for about 30 sec., about 72°C for about 1 min. for about 35 cycles; then clonal extension was performed at about 72°C for about 5 min.; PCR was performed in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, pH 3, 3 mM $MgCl_2$, about 1 unit Amplitaq Gold, about 0.1 μM dNTP's and about 0.4 μM primer. PCR products were resolved by electrophoresis on an about 1.2% LE agarose gel in TBE buffer and stained with ethidium bromide.

The results shown in Fig. 1A indicated that PCR products using cells isolated from the control dog were generated using all 7 Vβ specific primers. In addition, the levels of PCR products were substantially the same using the 7 Vβ specific primers. The results shown in Fig. 1B and 1C indicated that the Vβ profiles of PCR products using cells isolated from the lymphoma dog Haynes, or the lymphoma dog Stoll, were different from the Vβ profile of the control dog. In particular, T cells expressing hcVβ21 genes have been expanded in both patients, while T cells expressing the other 6 Vβ genes have decreased in proportion.

Taken together, the results disclosed in Examples 3 and 4 indicate that primers complementary to unique Vβ sequences of the present invention can be used to: (1) detect the presence of specific Vβ genes in a population of cells; (2) identify clonal expansion of cells expressing a particular Vβ gene; and (3) associate clonal T cell

expansion with an abnormal state or disease; or (4) distinguish a general lymphoproliferative state involving polyclonal T cell activation from clonal T cell expansion.

Example 6

This example describes a method for DNA fingerprinting the junctional regions of rearranged Vβ genes to identify clonal expansion of T cells.

DNA sequence analysis of the junction between the V, D and J regions of a Vβ gene can determine whether a mRNA population that translates into a Vβ protein is homogenous or heterogeneous by looking at the fluorescent DNA fingerprint. Fluorescent DNA fingerprints of Vβ mRNA populations were determined as follows. cDNA was prepared using standard methods from mitogen stimulated canine PBL cells isolated from a control dog and from lymph node cells isolated from the lymphoma dog Haynes or. The cDNA samples from either Haynes or the control dog were used as templates for PCR reactions using variable region primers in combination with constant region primers. Use of such primers were designed so that the resulting PCR products span the D/J junction of a Vβ cDNA. Primer hcVβ3 unique, primer hcVβ4 unique, primer hcVβ12 unique, primer hcVβ72 unique, primer hcVβ21 unique, primer dtb54 unique or primer dtb182 unique, was used in combination with the Cβ3 primer (SEQ ID NO:59). PCR reactions were performed using the following conditions: Taq activation was performed at about 95°C for about 10 min., about 94°C for about 30 sec., about 60°C for about 30 sec., about 72°C for about 1 min. for about 35 cycles; then clonal extension was performed at about 72°C for about 5 min.; PCR was performed in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, pH 3, 3 mM $MgCl_2$, about 1 unit Amplitaq Gold, about 0.1 μM dNTP's and about 0.4 μM primer.

About 20-25 μl of each resulting PCR product was resolved by electrophoresis on an about 1.2% LE agarose gel in TBE buffer and stained with ethidium bromide. DNA bands of about 400 bp identified on the gel were excised and the DNA purified. The purified DNA was sequenced using the Cβ2 primer (SEQ ID NO:58) using standard fluorescent dyes and an automated DNA sequencer.

Fluorescent electrophoretic histograms were generated for sequence obtained using each primer and each template. Fig. 2 illustrates 7 histograms generated using cDNA from the control dog and each of the 4 Vβ primers. The histograms show the typical heterogeneity of a T cell population in a normal dog. These histograms indicate that the DNA sequences of a particular amplified Vβ PCR product can be determined through the first about 120 bp of the Vβ constant region, but become ambiguous as the sequence profile enters the heterogenous J/D junctional regions.

Fig. 3 illustrates 4 histograms generated using cDNA from the lymphoma dog Haynes and Vβ primers hcVβ12 unique, hcVβ72 unique, hcVβ21 unique, dtb54 unique or dtb182 unique. The histograms show that 3 of the 4 Vβ genes amplified have fingerprints similar to those seen with a heterogeneous population of T cells, such as shown in Fig. 2. The histogram generated using the hcvb21 unique primer has a fingerprint which allows the unambiguous determination of the DNA sequence throughout the entire junctional region between the V, D, J and C regions. This result indicates that the sequence recognized by the hcvb21 unique primer was dominant among the canine PBL cell population from the lymphoma dog.

A comparison of the histogram generated using the hcvb21 unique primer with cDNA from the normal dog with the histogram generated using the hcvb21 unique primer with cDNA from the lymphoma dog is shown in Fig. 4. The comparison illustrates that the difference between the two histograms can be used to determine clonal expansion of a single T cell and association of such expansion to an abnormal state or disease.

Example 7

This example describes the generation of T cell clones reactive to flea saliva allergens, and the characterization of the TCR Vβ genes used by the T cell receptors of the T cell clones.

It is to be noted that this example includes a number of cellular immunology techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Coligan et al., *Current Protocol in Immunology,* Wiley Interscience, New York. T cell clones reactive to flea saliva

antigens were generated from peripheral blood mononuclear cells (PBMC) isolated from the experimentally-induced flea allergic dog CPO2 (described in U.S. Patent No. 5,646,115, issued on July 8, 1997) as follows. Blood samples were isolated from experimentally-induced flea allergic dog CPO2. Peripheral blood lymphocytes (PBL) were harvested from each sample by centrifugation using a ficoll gradient (available from Pharmacia). The PBL cells were cultured in about 5 ml cultures in culture medium containing IMDM and 10% fetal calf serum (available from Gibco, Gaithersburg, MD) at about 5 X 10^6 cells/ml in the presence of about 5 μg/ml flea saliva protein (prepared according to the methods described in U.S. Patent No. 5,646,115) for about 14 days, at about 37°C, in about 5% CO_2. The incubated cells were harvested and the number of viable cells determined. The viable cells were plated in 96 well round bottom plates at about 10^2 or about 10^3 cells per well per 200 μl of culture medium in the presence of about 10^5 autologous irradiated PBL (prepared according to the methods generally described in Coligan, *ibid.*) as antigen presenting cells (APC), about 5 μg/ml of flea saliva protein, and about 10 units/ml of recombinant hIL-2 for another 14 days. Wells that contain growing cells were restimulated *in situ* by replacing about 150 μl of spent culture medium (i.e., medium in which cells had been grown) with about 150 μl of fresh culture medium, flea saliva protein and APC as described above. About 10-14 days later, cultures in which the T cells were actively proliferating were transferred into 48 well plates, and tested for antigen specificity by comparing growth of the cells in the presence of flea saliva protein and APC, with growth of cells in the presence of APC alone. Cells that required flea saliva protein and APC to grow were selected and expanded in the presence of APC and flea saliva protein. These expanded cells are referred to as T cell clones.

Seven different T cell clones were derived from cells isolated from CPO2. mRNA was prepared from about 10^6 cells from each of the 7 clones using standard methods. cDNA was prepared from the mRNA using methods described above in Example 1. The cDNA samples from the 7 clones were then used as templates in PCR reactions to determine the presence of particular TCR Vβ molecules using the methods described above in Example 4. Analysis of the resulting PCR products indicated that 6

of the 7 clones expressed TCR using the dtb182 V beta chain, thereby indicating a bias in TCR Vβ usage in the T cell reactivity of CPO2 to flea saliva protein.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. An isolated protein comprising a protein selected from the group consisting of:

(a) an isolated protein having an amino acid sequence that is at least about 55 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 amino acids in length;

(b) an isolated protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 15 amino acids in length;

(c) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:35, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 25 amino acids in length; and

(d) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:38, SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 35 amino acids in length.

2. An isolated protein comprising a protein selected from the group consisting of:

(a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of

SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment that is at least about 30 nucleotides in length;

(c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

3. An isolated nucleic acid molecule having a nucleic acid sequence that is selected from the group consisting of:

(a) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:30 and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:33, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of

SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(c) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, SEQ ID NO:36, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:67, or a fragment thereof that is at least about 40 nucleotides in length;

(d) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:39, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length; and

(e) a nucleic acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

4. An isolated nucleic acid molecule selected from the group consisting of:

(a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30, or a fragment thereof, wherein said fragment has an at least a 20 contiguous nucleotide region identical in sequence to a 20 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, and SEQ ID NO:30;

(b) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, and SEQ ID NO:33, or a fragment thereof,

wherein said fragment has an at least a 25 contiguous nucleotide region identical in sequence to a 25 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31 and SEQ ID NO:33;

(c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36, or a fragment thereof, wherein said fragment has an at least a 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, and SEQ ID NO:36; and

(d) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39, or a fragment thereof, wherein said fragment has an at least a 60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, and SEQ ID NO:39.

5. An isolated nucleic acid molecule having a nucleic acid sequence encoding a protein selected from the group consisting of:

(a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of

SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

6. An isolated oligonucleotide comprising a unique nucleic acid sequence within a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID

NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, and SEQ ID NO:80; and a homolog thereof.

7. A reagent that is unique to a nucleic acid molecule selected from the group consisting of $nCaV\beta3_{381}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta3_{333}$, $nCaV\beta4_{351}$, $nCaV\beta12_{339}$, $nCaV\beta72_{423}$, $nCaV\beta21_{396}$, $nCaV\beta54_{354}$ and $nCaV\beta182_{369}$, wherein said reagent can distinguish one member of said group from another member of said group.

8. A method to detect expansion of T cells in an animal comprising:

(a) identifying the presence of one or more T cell receptor nucleic acid molecule(s) having unique nucleic acid sequences within variable regions of beta chain nucleic acid molecules selected from the group consisting of $nCaV\beta3_{381}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta3_{333}$, $nCaV\beta4_{351}$, $nCaV\beta12_{339}$, $nCaV\beta72_{423}$, $nCaV\beta21_{396}$, $nCaV\beta54_{354}$ and $nCaV\beta182_{369}$ by forming detectable products; and

(b) detecting the expansion of said T cells by determining production of said product.

9. A therapeutic composition that, when administered to an animal, regulates an immune response in said animal, said therapeutic composition comprising a therapeutic compound selected from the group consisting of:

(i) an isolated protein comprising a TCR Vβ protein selected from the group consisting of:

(a) an isolated protein having an amino acid sequence that is at least about 55 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 amino acids in length;

(b) an isolated protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 15 amino acids in length;

(c) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group

consisting of SEQ ID NO:10, SEQ ID NO:35, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 25 amino acids in length; and

(d) an isolated protein having an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:38, SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 35 amino acids in length;

(ii) a mimetope of any of said TCR Vβ proteins;

(iii) a chimeric form of any of said TCR Vβ proteins;

(iv) an isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:30 and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:33, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, and the complement of a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment thereof that is at least about 30 nucleotides in length;

(c) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:34, SEQ ID NO:36, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, and the complement

of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:67, or a fragment thereof that is at least about 40 nucleotides in length;

(d) a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:39, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, and the complement of a nucleic acid sequence that encodes an amino acid sequence selecte from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length; and

(e) a nucleic acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56;

(v) an isolated antibody that selectively binds to any of said TCR Vβ proteins; and

(vi) an inhibitor of TCR Vβ protein activity identified by its ability to inhibit the activity of said TCR Vβ proteins.

10. A method to produce a TCR Vβ protein, said method comprising culturing a cell capable of expressing said protein, said protein selected from the group consisting of:

(a) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:28, SEQ ID NO:50, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, or a fragment thereof that is at least about 25 nucleotides in length;

(b) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:31, SEQ ID NO:51, and a nucleic acid

sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, or a fragment that is at least about 30 nucleotides in length;

(c) a protein encoded by a nucleic acid molecule that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:52, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, or a fragment thereof that is at least about 40 nucleotides in length; and

(d) a protein encoded by a nucleic acid molecule that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:98, SEQ ID NO:17, SEQ ID NO:37, SEQ ID NO:53, and a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, or a fragment thereof that is at least about 75 nucleotides in length.

11. The invention of Claim 1, 2, 9 or 10, wherein said protein, when administered to an animal, can perform a function selected from the group consisting of eliciting an immune response against a TCR Vβ protein and binding to a MHC molecule that binds to a TCR Vβ protein.

12. The invention of Claim 1, 2, 9 or 10, wherein said protein is selected from the group consisting of: a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 and SEQ ID NO:71; and a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 and SEQ ID NO:71.

13. An isolated antibody that selectively binds to a protein as set forth in the invention of Claim 1, 2, 9 or 10.

14. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a TCR Vβ protein.

15. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule encodes a protein that elicits an immune response against a naturally-occurring TCR Vβ protein.

16. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule comprises a nucleic acid molecule selected from the group consisting of $nCaV\beta3_{381}$, $nCaV\beta4_{408}$, $nCaV\beta4_{384}$, $nCaV\beta12_{408}$, $nCaV\beta12_{402}$, $nCaV\beta72_{438}$, $nCaV\beta72_{399}$, $nCaV\beta3_{333}$, $nCaV\beta4_{351}$, $nCaV\beta12_{339}$ and $nCaV\beta72_{423}$.

17. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70 and SEQ ID NO:71; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences.

18. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17 and SEQ ID NO:18; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

19. The nucleic acid molecule of the invention of Claim 3-10, wherein said nucleic acid molecule comprises an oligonucleotide.

20. A recombinant molecule comprising a nucleic acid molecule as set forth in the invention of Claim 3-10 operatively linked to a transcription control sequence.

21. A recombinant virus comprising a nucleic acid molecule as set forth in the invention of Claim 3-10.

22. A recombinant cell comprising a nucleic acid molecule as set forth in the invention of Claim 3-10.

23. The invention of Claim 3-10, wherein said nucleic acid molecule comprises an oligonucleotide from about 15 nucleotides to about 25 nucleotides in length.

24. The invention of Claim 3-10, wherein said nucleic acid molecule comprises an oligonucleotide selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

25. The invention of Claim 3-10, wherein said nucleic acid molecule has a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

26. The invention of Claim 3-10, wherein said invention comprises a reagent which identifies the presence of a T cell receptor having a unique nucleic acid sequence within said nucleic acid molecule.

27. The invention of Claim 3-10, wherein said invention comprises a reagent which is a DNA primer complementary to said unique nucleic acid sequence.

28. The invention of Claim 3-10, wherein said invention comprises a unique nucleic acid sequence that is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and SEQ ID NO:56.

29. A kit comprising said reagent of Claim 7, wherein said kit comprises one or more of said reagents and a means for detecting said reagents.

30. A kit comprising said reagent of Claim 7, wherein said kit comprises seven of said reagents, wherein each of said seven reagents identifies the presence of a different beta chain V region selected from the group consisting of nCaV$\beta3_{381}$, nCaV$\beta4_{408}$, nCaV$\beta4_{384}$, nCaV$\beta12_{408}$, nCaV$\beta12_{402}$, nCaV$\beta72_{438}$, nCaV$\beta72_{399}$, nCaV$\beta3_{333}$, nCaV$\beta4_{351}$, nCaV$\beta12_{339}$, nCaV$\beta72_{423}$, nCaV$\beta21_{396}$, nCaV$\beta54_{354}$ and nCaV$\beta182_{369}$.

31. The kit of Claim 30, wherein said kit further comprises a DNA primer complementary to a nucleic acid sequence in the constant region of a T cell receptor beta chain.

32. The kit of Claim 30, wherein said constant region nucleic acid sequence is selected from the group consisting of SEQ ID NO:58 and SEQ ID NO:59.

33. The kit of Claim 30, wherein said kit comprises a composition comprising a mixture of said reagents and said DNA primer complementary to a nucleic acid sequence in the constant region of a T cell receptor beta chain.

34. The method of Claim 8, wherein said step of detecting comprises comparing formation of one detectable product with one or more other detectable products.

35. The method of Claim 8, wherein said variable region has a nucleic acid sequence selected from the group consisting of SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and complements of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

36. The method of Claim 8, wherein said step of identification comprises:

(a) contacting a sample containing DNA from T cells with a reagent having specificity for one of said unique nucleic acid sequences; and

(b) determining the presence of DNA carrying said unique nucleic acid sequence.

37. The method of Claim 8, wherein said reagent is a DNA primer that is complementary to said unique nucleic acid sequence.

38. The method of Claim 8, wherein said step of identification is performed using polymerase chain reaction amplification.

39. The method of Claim 8, wherein said method detects a disease that is selected from the group consisting of cancer, an autoimmune disease, an infectious disease and allergy.

40. The invention of Claim 8 or 9, wherein said animal is selected from the group consisting of an animal suspected of having a disease, an animal having a disease and an animal being treated for a disease, wherein said disease is selected from the group consisting of lymphoma, leukemia, rheumatoid arthritis, diabetes, viral infections, bacterial infections, yeast infections, parasite infections, dermatitis, and asthma.

41. The method of Claim 40, wherein said disease is selected from the group consisting of T cell lymphoma and T cell leukemia.

42. A composition comprising the invention of Claim 1, 2, 3, 4, 5, 6, or 9, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant and a carrier.

43. The composition of Claim 9, wherein said therapeutic compound is selected from the group consisting of a peptide, a naked nucleic acid vaccine and a recombinant cell vaccine.

44. A method to regulate an immune response in an animal comprising administering to the animal a therapeutic composition comprising the invention of Claim 1, 2, 3, 4, 5, 6, or 9.

45. A method for prescribing treatment for specific disease, comprising:

(a) identifying the presence of a T cell receptor nucleic acid molecule having a unique nucleic acid sequence within a variable region of a beta chain nucleic

acid molecule selected from the group consisting of nCaV$\beta 3_{333}$, nCaV$\beta 4_{351}$, nCaV$\beta 12_{339}$, nCaV$\beta 72_{423}$, nCaV$\beta 21_{396}$, nCaV$\beta 54_{354}$, nCaV$\beta 182_{369}$, nCaV$\beta 3_{381}$, nCaV$\beta 4_{408}$, nCaV$\beta 12_{408}$, nCaV$\beta 72_{438}$, nCaV$\beta 21_{462}$, nCaV$\beta 54_{417}$ and nCaV$\beta 182_{423}$; and

(b) prescribing a treatment comprising administering to said animal a therapeutic composition comprising the invention of Claim 1, 2, 3, 4, 5, 6, or 9.

46. The reagent of Claim 7, wherein said reagent identifies the presence of a T cell receptor having a unique nucleic acid sequence within said nucleic acid molecule.

47. The reagent of Claim 7, wherein said reagent is a DNA primer complementary to said unique nucleic acid sequence.

48. The reagent of Claim 7, wherein said unique nucleic acid sequence is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and SEQ ID NO:56.

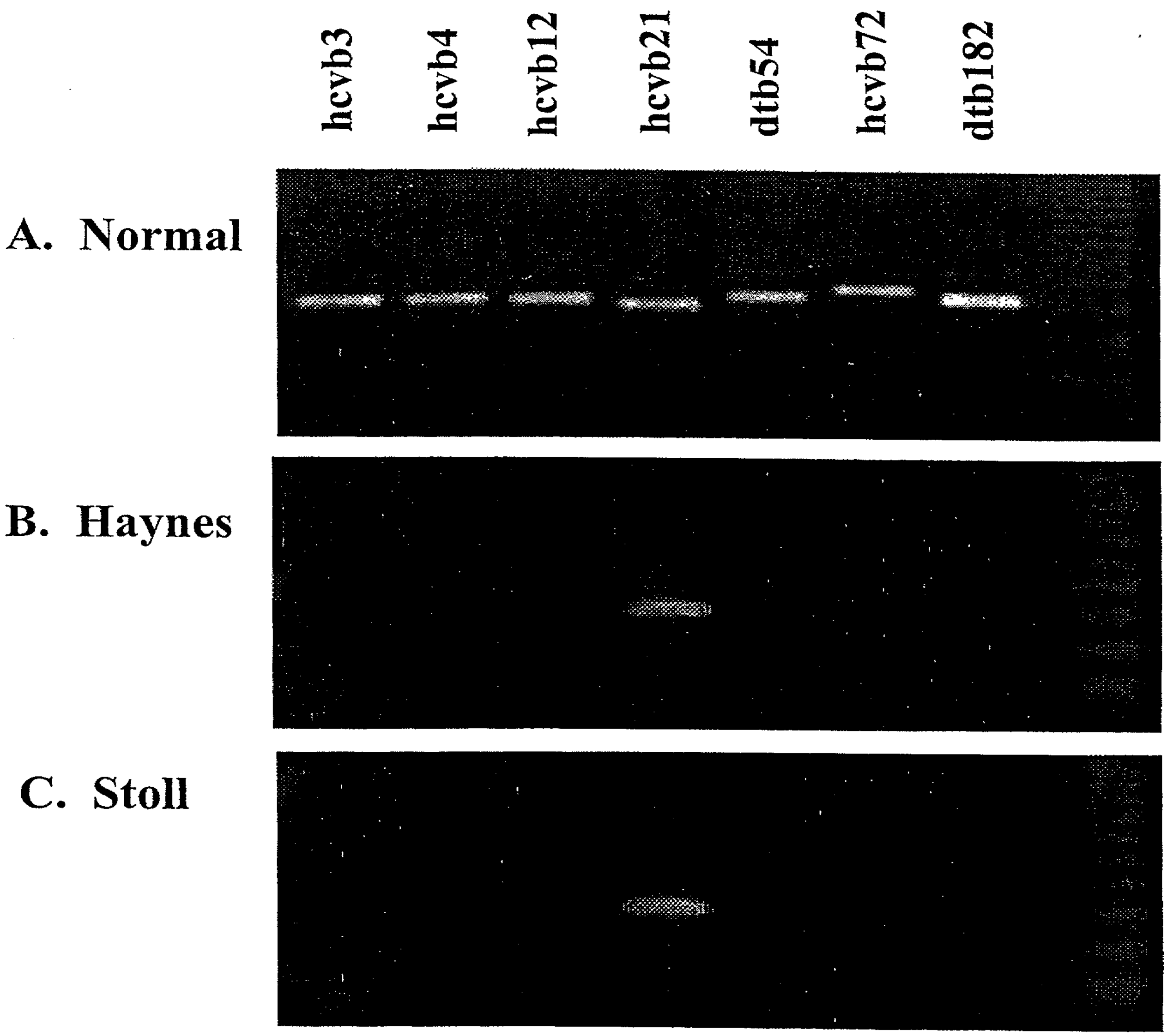
hcvb3
hcvb4
hcvb12
hcvb21
dtb54
hcvb72
dtb182
A. Normal
B. Haynes
C. Stoll

Fig. 1

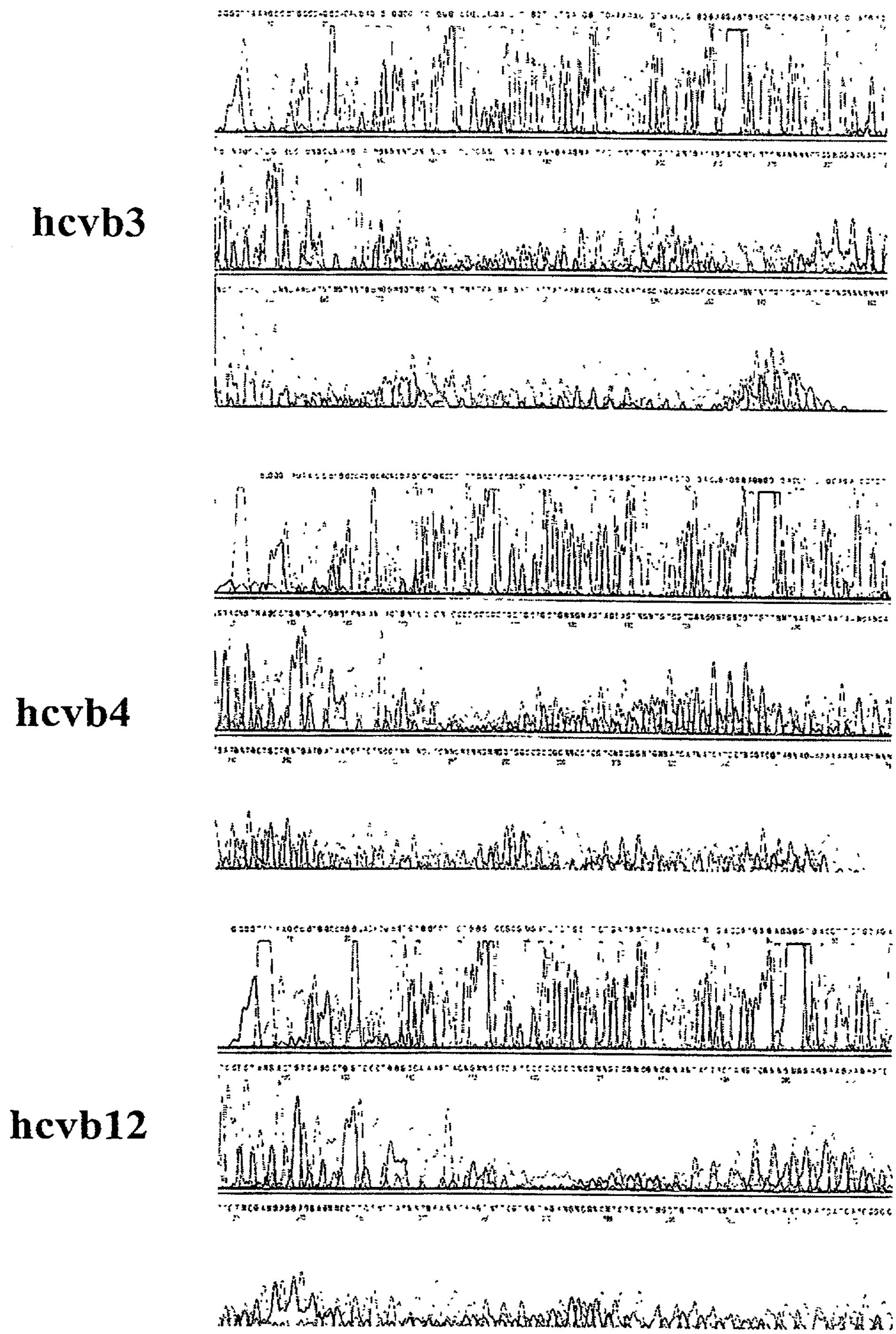

Fig. 2A

hcvb21

dtb54

hcvb72

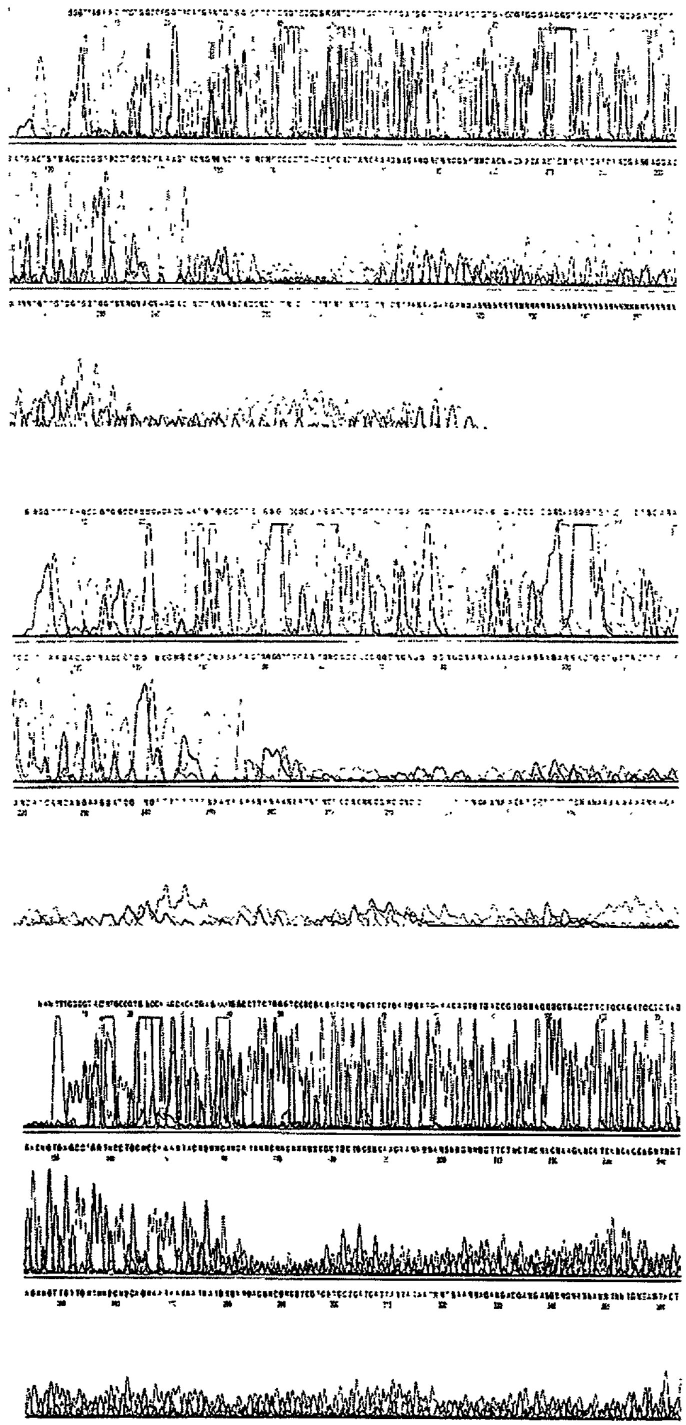

Fig. 2B

dtb182

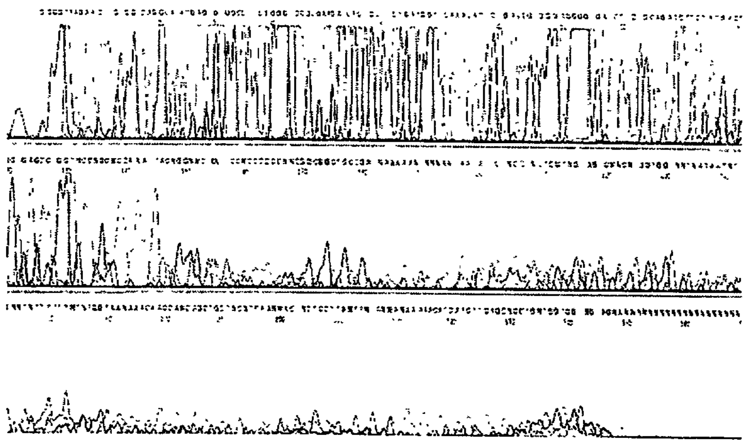

Fig. 2C

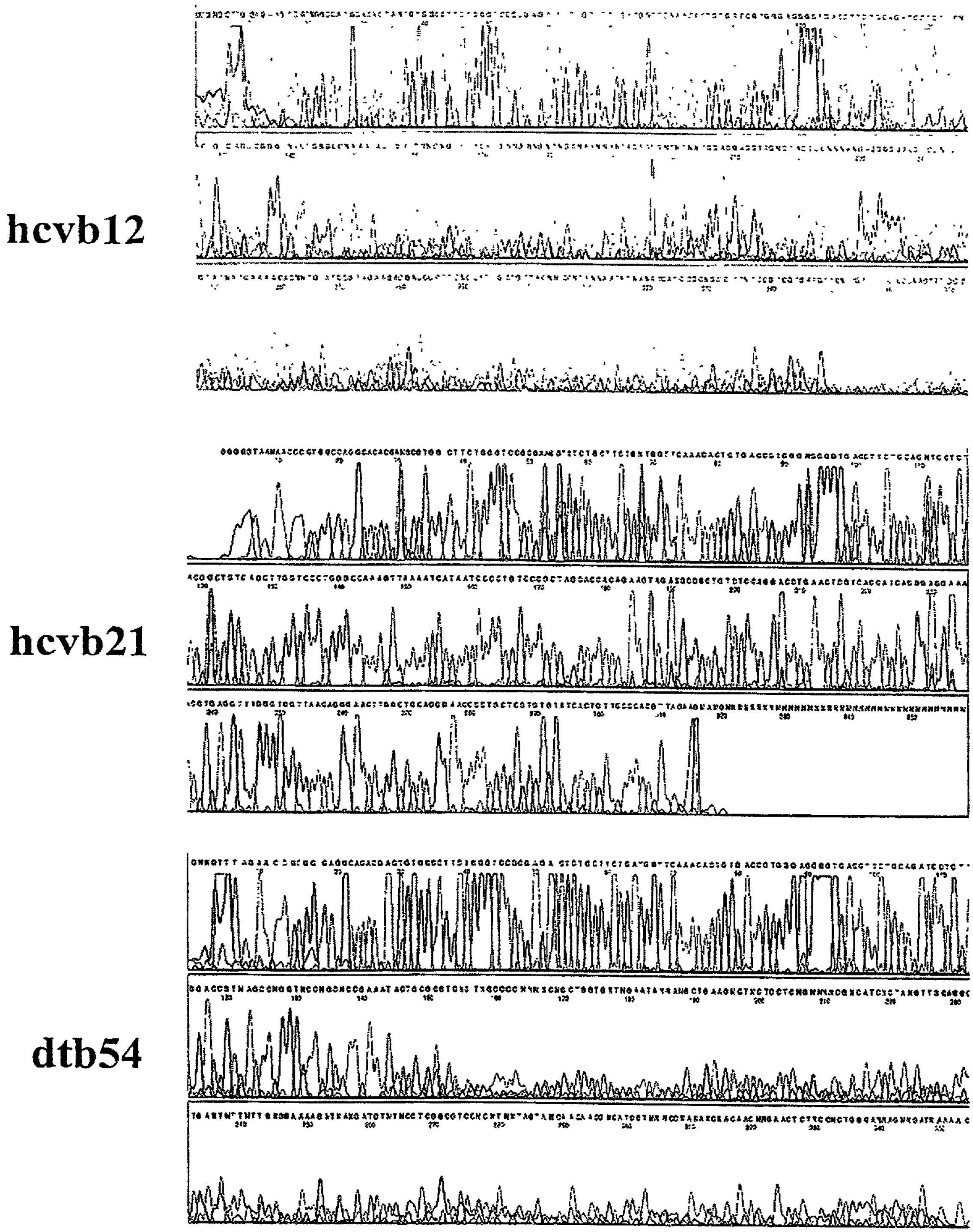

Fig. 3A

dtb182

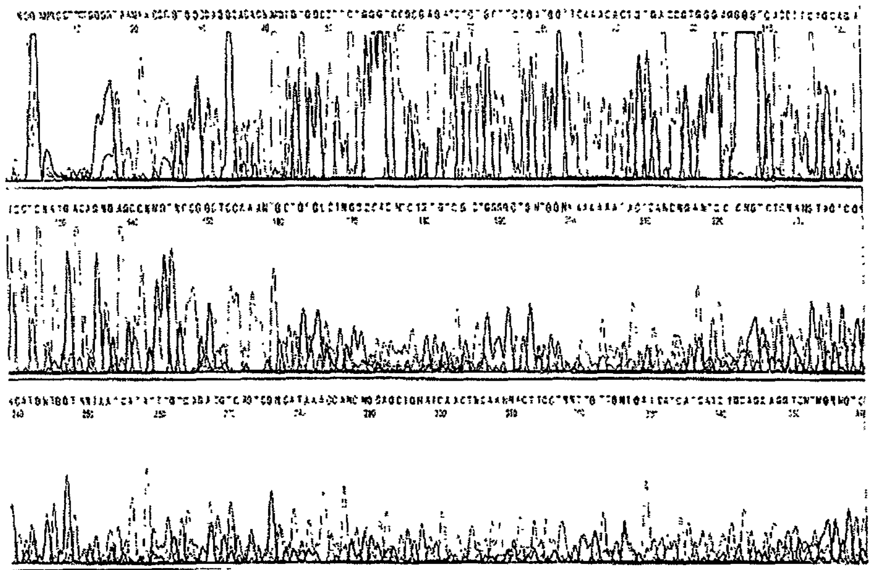

Fig. 3B

Normal
hcvb21

Haynes
hcvb21

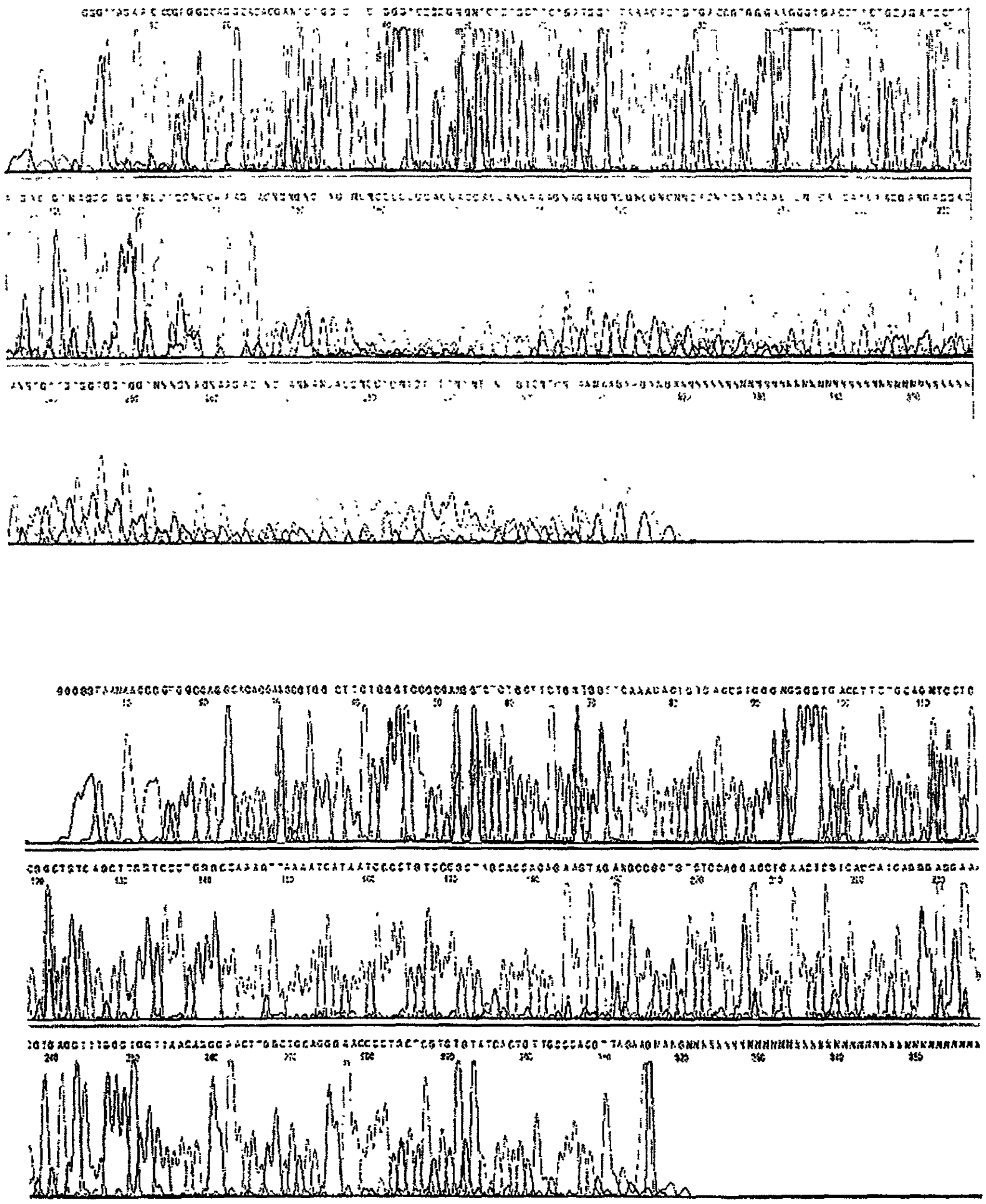

Fig. 4

OIPE
JUL 0 5 2001
PATENT & TRADEMARK OFFICE

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit:
Sim, Gek-Kee Dreitz, Matthew	Examiner:
Serial No.: 09/744,847	**DECLARATION OF SUSAN GORDON**
Filed: January 29, 2001	
Atty. File No.: 2618-102-PUS	
For: "T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"	

Commissioner for Patents
Washington, DC 20231

Dear Sir:

I, Susan Gordon, have firsthand knowledge of the facts set forth in paragraphs 1-8 below.

1. Gek-Kee Sim is a joint inventor of the above-identified patent application and a former employee of Heska Corporation.

2. Dr. Sim was an employee at Heska Corporation from February 1, 1996 through August 25, 1998. (A copy of Dr. Sim's "Employee Confidential Information and Inventions" agreement is attached hereto as Exhibit A.) The last known official address provided by Dr. Sim that Heska Corporation has on file is 3622 Terry Point Drive, Fort Collins, CO 80524. However, since that time, we have determined that Dr. Sim's current address is 543 Franklin Street, Denver, CO 80218-3623 (see below).

3. On July 23, 1999, I met with Dr. Sim at the Starbuck's on Drake Avenue in Fort Collins, CO to get her signature on patent documents unrelated to the present application. Dr. Sim informed me that she was putting her 3622 Terry Point Drive, Fort Collins, CO 80524 residence up for sale, and planned to move to the house of her acquaintance Dr. Andre Augustine.

4. On July 30, 1999, a spiral-bound copy of the specification and claims as filed for PCT/US99/17309 (on which the present application is based) was sent to Dr. Sim at the last known official address on Terry Point Drive in Fort Collins.

5. On December 9, 1999, Carol Talkington Verser, Ph.D. (an in-house patent agent at Heska Corporation), sent by Federal Express a spiral-bound copy of an entire patent application (including specification and claims) and documents for execution, unrelated to this case, to Dr. Sim at the last known official address on Terry Point Drive in Fort Collins. When we did not hear back from Dr. Sim, we resent the package of documents to her on April 19, 2000, to the address of Dr. Augustine on Franklin Street in Denver. The FedEx package was delivered to and signed for by A. Augustine, an acquaintance of Dr. Sim, but Dr. Sim did not respond. Dr. Verser sent another letter by e-mail to Dr. Sim in April 2000, but did not receive a response to the letters or e-mails. A copy of these documents is attached as Exhibit B.

6. In March of 2000, I called the Fort Collins Post Office and spoke with Steve O'Connell asking if Dr. Sim had left forwarding address information. Mr. O'Connell said that mail addressed to Dr. Sim at 3622 Terry Point Drive, Fort Collins, CO 80524 is not forwarded. Dr. Sim is no longer at that address and left no forwarding order.

7. On April 5, 2000, I sent a letter, enclosing a manuscript, signed by Carol Talkington Verser addressed to Dr. Sim, c/o 543 Franklin Street, Denver, CO 80218-3623 (Dr. Augustine's address), asking for her review and signature of a manuscript. Dr. Sim signed the enclosure to that letter, "Heska Technical Presentation Approval Form," and returned it with a note. Copies of the letter, Dr. Sim's signature on the approval form and Dr. Sim's note, along with the envelope in which she returned them, are attached as Exhibit C. Please note that the return address on the envelope used by Dr. Sim indicates that Dr. Sim is now using 543 Franklin Street, Denver, CO, as her return address.

8. On December 22, 2000, I sent a letter by Certified Mail on behalf of Carol Talkington Verser, Ph.D. to Dr. Sim at 543 Franklin Street, Denver, Colorado, enclosing a copy of the Declaration and Assignment for the above-referenced patent application requesting that she sign the documents and return them to Dr. Verser in the return FedEx package provided. To date we have not received the signed documents, nor have we received the Certified letter return receipt requested. A copy of the Certified letter is attached as Exhibit D.

Several times, I have called the claims office of the U.S. Post Office Capitol Hill Station in Denver, Colorado, asking for information on the Certified letter mailed December 22, but they

have been unsuccessful in tracing the letter. Their policy is to leave the addressee a first notice of the Certified letter. When the Certified letter is unclaimed, the mail carrier delivers a second notice five days after the first notice. The post office cannot trace the letter until it has been at least 21 days since the date of the first notice. The claims office has been unable to track this particular certified letter.

I hereby declare that all statements made herein of my own are true and that statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Dated: June 27, 2000

Susan Gordon

Susan Gordon
Manager, Intellectual Property Administration, for
Carol Talkington Verser, Ph.D. (Reg. No. 37,459)
Heska Corporation
1613 Prospect Parkway
Fort Collins, Colorado 80525
Telephone: (970) 493-7272
Facsimile: (970) 491-9976

PTO/SB/01 (6-95)
Approved for use through 10/31/98 OMB 0651-0032

Type a plus sign (+) inside this box → ☐

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

O I P E JUL 0 5 2001 PATENT & TRADEMARK OFFICE

DO/PTO Rev 6/95 U.S. Department of Commerce Patent and Trademark Office	Attorney Docket Number	2618-102-PCT
DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION	First Named Inventor	SIM, Gek-Kee
	COMPLETE IF KNOWN	
	Application Number	
	Filing Date	
☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing	Group Art Unit	
	Examiner Name	

As below named inventor, I hereby declare that::

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed for which a patent is sought on the invention entitled:

"T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"

(*Title of the Invention*)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 29 July 1999 as United States Application Number or PCT International

Application Number PCT/US99/17309 and was amended on (MM/DD/YYYY) ______ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 (a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any Pct international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				Yes	No
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)		
60/094,506	July 29, 1998	☐	Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

Burden Hour Statement: This form is estimated to take .4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information officer, Patent and Trademark Office, Washington, DC 20231.
DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents Washington, DC 20231

Type a plus sign (+) inside this box → ☐

DECLARATION	Page 2

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States of PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112.1 acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (*if applicable*)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☒ Firm Name OR: SHERIDAN ROSS P.C. — Customer Number or label: ☐

☐ List attorney(s) and/or agent(s) name and registration number below:

Name	Registration Number	Name	Registration Number
ZINGER, DAVID F.	29,127	STAVISH, SABRINA CROWLEY	33,374
GROSETH, CRAIG C.	31,713	HANSEN, LEWIS D.	35,536
TOMPKINS, MICHAEL L.	30,980	KOVARIK, JOSEPH E.	33,005
BLAKELY, TODD P.	31,328	SWARTZ, DOUGLAS W.	37,739
CONNELL, GARY J	32,020	KUGLER, BRUCE A.	38,942
CROOK, WANNELL M.	31,071	BRUNELLI, ROBERT R.	39,617

12

☒ Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto

Please direct all correspondence to: ☐ Customer Number or label | OR ☐ Fill in correspondence address below

Name	GARY J. CONNELL				
Address	1560 BROADWAY, SUITE 1200				
Address					
City	DENVER	State	COLORADO	Zip	80202-5141
Country	UNITED STATES OF AMERICA	Telephone	(303) 863-9700	Fax	(303) 863-0223

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name	Gek-Kee	Middle Initial		Family Name	SIM	Suffix e.g. Jr.	
Inventor's Signature				Date			
Residence City	Fort Collins	State	Colorado	Country	USA	Citizenship	USA
Post Office Address	3622 Terry Point Drive						
Post Office Address							
City	Fort Collins	State	Colorado	Zip	80524	Country	USA
Applicant Authority							

☐ Additional inventors are being named on supplemental sheet(s) attached hereto.

Type a plus sign (+) inside this box → ☐

# DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name	Matthew	Middle Initial	J	Family Name	DREITZ	Suffix e.g. Jr.	
Inventor's Signature				Date	12/28/00		
Residence City	Fort Collins	State	Colorado	Country	USA	Citizenship	USA
Post Office Address	~~4324 Winterstone~~						
Post Office Address	6187 View Point Ave.						
City	~~Fort Collins~~ Firestone	State	~~Colorado~~ CO	Zip	~~80525~~ 80504	Country	~~USA~~ USA
Applicant Authority							

Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature				Date			
Residence City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
Applicant Authority							

Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature				Date			
Residence City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
Applicant Authority							

Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature				Date			
Residence City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
Applicant Authority							

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet

Type a plus sign (+) inside this box → ☐

DECLARATION	ADDITIONAL and/or AGENT INFORMATION Supplemental Sheet

Name	Registration Number	Name	Registration Number
HUGHES, RICHARD L.	31,264		
HANSRA, TEJPAL S.	38,172		
CARDWELL, DANA HARTJE	40,638		
DALLAS-PEDRETTI, ANGELA	42,460		
LIEB, BENJAMIN B.	42,801		
KNEPPER, BRADLEY M.	44,189		
TRUDELL, MIRIAM DRICKMAN	42,499		
DuPRAY, DENNIS J.	46,299		
PETERSEN, Todd M.	45,580		
WINTERTON, Kenneth C.	48,040		
TRAVER, Robert D.	47,999		
JOHNSON, Brent P.	38,031		
YASKANIN, Mark L.	45,246		

I hereby declare that the attached "EMPLOYEE CONFIDENTIAL INFORMATION AND INVENTIONS AGREEMENT" is a true copy of the agreement signed by Gek-Kee Sim governing her employment at Heska Corporation from February 1, 1996 through August 25, 1998, which assigns all right, title and interest in all Inventions made by Gek-Kee Sim, as defined in Section 2 of the Agreement, to Heska Corporation.

By: Miroslawa Saraullo
Name: Miroslawa Saraullo
Title: HR Representative

STATE OF COLORADO)
) ss.
COUNTY OF LARIMER)

Before me, a Notary Public in and for said county and state, on this 26th day of ~~April~~ January ~~1999,~~ 2001 personally appeared Miroslawa Saraullo, as HR Representative of Heska Corporation, 1613 Prospect Parkway, Fort Collins, Colorado, known to me to be the person whose name is subscribed to the foregoing instrument.

Susan A. Gordon
Notary Public

My commission expires: Nov. 22, 2004.

Exhibit A

COPY

EMPLOYEE CONFIDENTIAL INFORMATION AND INVENTIONS AGREEMENT

THIS AGREEMENT is between Heska Corporation a California corporation (the"Company"), and GekKee Sim (Employee").

PURPOSE OF THE AGREEMENT

I wish to be employed by, or continued in the employment of, the Company, and the Company wishes to employ me, or continue to employ me, provided that, in so doing, it can protect its trade secrets and inventions, ideas, information, business, and good will.

In consideration of this purpose, and the mutual promises in this Agreement, I agree with the Company as follows:

1. <u>Confidential Information</u>. I will hold in confidence during the entire term of my employment and for five years after the termination of my employment all Confidential Information of the Company and all Confidential Information of companies or persons other than the Company given to the Company under an agreement prohibiting its disclosure. "Confidential Information" refers to valuable technical or business information that is not known by the public. By way of example, Confidential Information may include information relating to: inventions or products, including unannounced products; research and development activities; requirements and specifications of specific customers and potential customers; nonpublic financial information; and quotations or proposals given to customers.

These restrictions on disclosure do not apply if (i) the information is or becomes publicly known through no wrongful act or part; (ii) I already knew the information prior to the time I began employment with the Company, other than by disclosure to me by the Company; (iii) I received the information without any wrong doing from someone outside the Company who does not have an obligation to keep the information confidential; (iv) the information is explicitly approved for release by an officer of the Company; or (v) the information is disclosed pursuant to the requirement of a governmental agency.

(2) <u>Disclosure and Assignment of Inventions</u>. I will promptly disclose and assign to the Company my entire right, title and interest in all Inventions. "Inventions" refers to (a) all technical or business innovations, whether or not patentable or copyrightable, made by me during the term of my employment; and (b) all technical or business innovations, whether or not patentable or copyrightable, based upon the Company's Confidential Information and made by me after leaving the Company's employment. I will keep adequate written records of all Inventions made by me, such as notebooks, sketches, program listings and the like, which are the property of the Company. Notwithstanding the foregoing, I am not required to assign to the Company, although I must disclose, any invention: (a) for which no equipment, supplies, facilities or Confidential Information of the Company were used and which was developed entirely on my own time; (b) which at the time of conception or reduction to practice did not relate directly to the business of the Company or the Company's actual or demonstrably anticipated research or development; and (c) which did not result from any work I performed for the Company. The disclosure of such inventions must be made so that the parties can make a determination whether such inventions do in fact qualify for exclusion from assignment to the Company. The Company will keep confidential any such information I disclose. I will take all steps necessary to assist the Company in securing any patents, copyrights, or other protection for Inventions which I am required to assign to the Company as provided above. If I am unable or unwilling, whether during my employment or after termination, to sign any papers needed to apply for or pursue any patent or

copyright registrations for Inventions, I agree that the Company is my attorney-in-fact for that purpose and can sign such papers as my agent and take any other actions necessary to pursue these registrations.

3. Tangible Materials. All tangible materials that incorporate Confidential Information are the Company's property, and I will give all these materials back to the Company at the termination of my employment or earlier upon the Company's request.

4. Duties to Third Parties. I represent that, to the best of my knowledge, compliance with the terms of this Agreement will not violate any duty that I may have to anyone other than the Company (such as a former employer) to keep such person's proprietary information in confidence or to refrain from using that person's patents or copyrights. If at any time during my employment with the Company, I am asked by the Company to perform work which I believe may cause me to violate a duty I have to someone other than the Company, I will immediately inform an officer of the Company so that an assessment of the situation may be made. I also agree that I will not, during my employment with the Company, bring onto the Company's premises, use or disclose to the Company any proprietary information or trade secrets of any former employer or any other person without that person's consent.

5. Miscellaneous. This is the only agreement between the Company and myself about confidential information and the ownership of inventions, and may not be modified, amended or terminated, in whole or in part, except in a writing signed by me and by an officer of the Company. I agree that, unless it is so modified, amended, or terminated, this Agreement shall be operative and binding with respect to the entire term of my employment with the Company, even if such term or employment commenced prior to the date on which I signed this Agreement. Any change in my title, compensation or duties will not affect this Agreement. This Agreement, other than the provisions which are expressly applicable only during my employment with the Company, will survive termination of my employment for any reason, and will continue for the benefit of and will be binding upon the successors, assigns, heirs and legal representatives of the Company and myself. The waiver by the Company of a breach of any of the obligations of this Agreement by me will not operate or be construed as a waiver of any other or subsequent breach by me. In the event any provision of this Agreement is held to be invalid, void or unenforceable, the remaining provisions will nevertheless continue in full force and effect without being impaired or invalidated in any way. This Agreement will be governed by the laws of the State of California governing contracts between residents to be performed in the State of Colorado.

HESKA CORPORATION

Fred M. Schwarzer
President/CEO

Signature

EMPLOYEE

Signature

Gordon, Susan

From: Gordon, Susan
Sent: Wednesday, December 01, 1999 3:03 PM
To: 'Gek-Kee Sim'
Subject: new patent filing

Hi Gek-Kee,

I hope things are going well for you in Denver. We're filing a new patent application entitled . You are an inventor on this new continuation-in-part application, and I will need to send you an assignment and declaration for signature. Would you send me your new address and telephone number so I can make that change on our records?

Thanks, and I hope to hear from you soon.

Susan

Exhibit B

December 9, 1999

HESKA

1613 Prospect Parkway
Fort Collins, CO 80525
(t) 970-493-7272
(f) 970-493-7333
www.heska.com

Gek-Kee Sim, Ph.D.
3622 Terry Point Drive
Fort Collins, Colorado 80524

RE: U.S. Patent Application entitled,

Dear Gek-Kee, Shumin and Matt:

Please find enclosed a spiral-bound copy of the above-referenced patent application which was filed with the U.S. Patent and Trademark Office on This filing is a continuation-in-part application of a U.S. Patent Application filed , entitled ; which claims priority to a U.S. Provisional Patent Application filed , entitled Because this application contains Heska confidential information, we ask that you keep the application and related documents in a secure place.

Also enclosed are a Declaration and an Assignment which we will need to file in the next month or two in support of the above-referenced patent application. Please sign and return these forms to us in the attached return FedEx envelope. Please note that your signature on the Assignment needs to be notarized. If your address has changed, please cross out the wrong address, print your correct address, and initial this change.

Thank you.

Sincerely,

Carol Talkington Verser

Carol Talkington Verser, Ph.D.
Vice President, Intellectual Property

Encls.

Gordon, Susan

From: Verser, Carol
Sent: Wednesday, April 19, 2000 10:15 AM
To: 'Gk2463622@aol.com'
Cc: Gordon, Susan
Subject: Papers for signature for patent application

Dear Gek-kee,

I hope everything is going well for you.

Today, April 19, we are sending to you via FedEx a Declaration and Assignment on which we need your signature for a continuation-in-part patent application entitled ', filed . We also enclosed a return FedEx package. We would really appreciate it if you could sign and return those papers by May 12, 2000, since we need to file the papers with the patent office before May 18, 2000. Please let me know if you have any questions about this filing.

Thanks, and take care!
Carol

HESKA
1613 Prospect Parkway
Fort Collins, CO 80525
(t) 970-493-7272
(f) 970-493-7333
www.heska.com

April 19, 2000

Via Federal Express

Gek-Kee Sim, Ph.D.
c/o 543 Franklin Street
Denver, Colorado 80218-3623

RE: U.S. Patent Application entitled,

filed
(Our patent file no.:)

Dear Gek-Kee:

Please find enclosed, for execution by you, a Declaration and an Assignment which we need to file before , in support of the above-referenced patent application. Please sign and return these forms to us in the attached return FedEx envelope. Please note that your signature on the Assignment needs to be notarized. If your address has changed, please cross out the wrong address, print your correct address, and initial this change.

On December 9, 1999, we mailed to you a spiral-bound copy of the above-referenced patent application. We are assuming you received it because it never came back. If you do not have a copy of the application as filed, please let me know and we will send another. This filing is a continuation-in-part application of a U.S. Patent Application filed), entitled

; which claims priority to a U.S. Provisional Patent Application filed entitled

. Because this application contains Heska confidential information, we ask that you keep the application and related documents in a secure place.

Thank you.

Sincerely,

Carol Talkington Verser

Carol Talkington Verser, Ph.D.
Vice President, Intellectual Property

Encls.

FedEx® Letter
eral Express

Weight Limit: 8 Ounces

mitation on Contents

Declared Value/
Liability Information

For More Information

For more information please contact your local

FedEx USA Airbill

FedEx Tracking Number 801429352118

0200 Form ID No.

SPL11

Sender's Copy

1 From (please print and press hard)

Date 4- -00 Sender's FedEx Account Number

Sender's Name Gek-Kee Sim, Ph.D. Phone ( )

Company

Address 543 Franklin St. Dept./Floor/Suite/Room

City Denver State CO ZIP 80218-3623

2 Your Internal Billing Reference Information (Optional) (First 24 characters will appear on invoice) 1410

3 To (please print and press hard)

Recipient's Name Carol Talkington Verser, Ph.D. Phone (970) 493-7272

Company HESKA CORP

Check here if residence (Extra charge applies for FedEx Express Saver)

Address 1613 PROSPECT PARKWAY
(To "HOLD" at FedEx location, print FedEx address here) (We Cannot Deliver to P.O. Boxes or P.O. ZIP Codes) Dept./Floor/Suite/Room

City FORT COLLINS State CO ZIP 80525

For HOLD at FedEx Location check here
- Hold Weekday (Not available with FedEx First Overnight)
- Hold Saturday (Not available at all locations) (Available for FedEx Priority Overnight and FedEx 2Day only)

For Saturday Delivery check here
- (Extra Charge. Not available to all locations) (Available for FedEx Priority Overnight and FedEx 2Day only)

4a Express Package Service *Packages under 150 lbs.* Delivery commitment may be later in some areas
- FedEx Priority Overnight (Next business morning)
- [X] FedEx Standard Overnight (Next business afternoon)
- FedEx 2Day* (Second business day)
- FedEx Express Saver* (Third business day)
- FedEx First Overnight (Earliest next business morning delivery to select locations) (Higher rates apply)

*FedEx Letter Rate not available. Minimum charge One pound rate

4b Express Freight Service *Packages over 150 lbs.* Delivery commitment may be later in some areas
- FedEx Overnight Freight (Next business day)
- FedEx 2Day Freight (Second business day)
- FedEx Express Saver Freight (Up to 3 business days)

(Call for delivery schedule. See back for detailed descriptions of freight services)

5 Packaging
- [X] FedEx Letter (Declared value limit $500)
- FedEx Pak
- FedEx Box
- FedEx Tube
- Other Pkg

6 Special Handling
Does this shipment contain dangerous goods?
- Yes (As per attached Shipper's Declaration)
- Yes (Shipper's Declaration not required)
- Dry Ice (Dry Ice, 9, UN 1845 III x kg 904) (Dangerous Goods Shipper's Declaration not required)
- CA Cargo Aircraft Only

7 Payment
Bill to:
- Sender (Account no. in section 1 will be billed)
- Recipient
- [X] Third Party
- Credit Card
- Cash/Check

(Enter FedEx account no. or Credit Card no. below)

FedEx Account No. 1240-2891-4

Credit Card No. Exp Date

Total Packages	Total Weight	Total Declared Value*	Total Charges
		$ .00	$

*When declaring a value higher than $100 per shipment, you pay an additional charge. See SERVICE CONDITIONS, DECLARED VALUE, AND LIMIT OF LIABILITY section for further information.

8 Release Signature Sign to authorize delivery without obtaining signature

Your signature authorizes Federal Express to deliver this shipment without obtaining a signature and agrees to indemnify and hold harmless Federal Express from any resulting claims.

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REQUIRED INFORMATION
Sent By: CTV Ext: 4116 Group Number: 1410

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Does this package require **insurance**? ☐ Yes ☒ No

If yes, amount: ____

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If yes, name and quantity:

Name: Quantity:

Would you like this package released without a signature? ☐ Yes ☒ No

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Is this package a loaner or demo return? ☐ Yes ☒ No

Is this package a warranty return or repair? ☐ Yes ☒ No

Is this package a return to a supplier? ☐ Yes ☒ No

RGA #:

Item #:

Description:

Quantity:

P.O. #:

SHIP TO:

Attention: Gek-Kee Sim, Ph.D.

Company:

Street: 543 Franklin St.

City/State/Zip Denver, CO 80218-3623

Phone #:

METHOD OF SHIPMENT: (Check One)

☒ Fed Ex Priority Overnight (10:30 AM)
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041900

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1) The commercial value and
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April 5, 2000

CERTIFIED MAIL
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Gek-Kee Sim, Ph.D.
c/o 543 Franklin Street
Denver, Colorado 80218-3623

Dear Gek-Kee:

Enclosed please find a copy of a manuscript entitled "

with the following authors: Shumin Yang, Karen Sellins, Tim Powell, Earl Stoneman, and Gek Kee Sim. We welcome any suggestions that you may have to this manuscript, and encourage you to send them to me. As you are aware, it is Heska's policy that all authors must read and approve the contents of any manuscripts with which they are associated. We are also sending you a copy of the Heska Technical Presentation Approval Form for you to sign. We would like to submit this manuscript as soon as possible. If we do not hear from you by April 26, 2000, we will assume that you do not wish to be an author on this paper and remove your name from it.

I hope all is well with you.

Sincerely,

Carol Talkington Verser

Carol Talkington Verser, Ph.D.
Vice President, Intellectual Property

Encl.
Copy to: Karen Sellins, Ph.D.
Donald Wassom, Ph.D.

Exhibit C

P 160 589 515 Manuscript

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TITLE: ____

PRINCIPAL AUTHOR: Shumin Yang

OTHER CO-AUTHORS: Karen Sellins, Tim Powell, Earl Stoneman,.Gek Kee Sim

Approved by:

[signature] Principal Author	1/16/00 Date
Karen Sellins Co-Author	2/23/00 Date
Tim Powell Co-Author	3/17/00 Date
[signature] Co-Author	4/23/00 Date
Project Coordinator	Date
Chief Scientific Officer	Date
Vice President, Intellectual Property	Date

For subjects related to clinical, development, and regulatory matters:

Executive Vice President	Date

4/23/00

Dear Carol,

The manuscript is O.K. I would prefer to use feline instead of cat in the title, and canine instead of dog in the abstract &

Best Regards

Gek Kee

Gek-Kee Sim
543 Franklin St.
Denver CO 80218

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December 22, 2000

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Gek-Kee Sim, Ph.D.
543 Franklin Street
Denver, Colorado 80218

RE:

National Phase Filings of International CT Patent Application No. PCT/US99/17309 entitled "T CELL RECEPTOR PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"
Our File No. IM-3-C1-PCT (2618-102-PCT)

Dear Gek-Kee:

Please find enclosed the following documents for signature by you as an inventor on the above-referenced patent applications:

•

• an "Assignment" and "Declaration for Utility or Design Patent Application" for the National Phase Filings of International PCT Patent Application No. PCT/US99/17309.

These documents were sent to us by outside counsel, who is handling prosecution of these applications. We would appreciate it if you would sign and date these documents and return them to us in the attached Federal Express package at your earliest convenience and, if at all possible, before December 29, 2000. If you need to make a correction to your name or address, please cross through the wrong items, print the correct information and initial the change. Also, please note that your signature needs to be notarized on the Assignment. If you have any questions, please feel free to call me. Thank you.

Sincerely,

Carol Talkington Verser/sg

Carol Talkington Verser, Ph.D.
Vice President, Intellectual Property and
Business Development

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Exhibit D

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